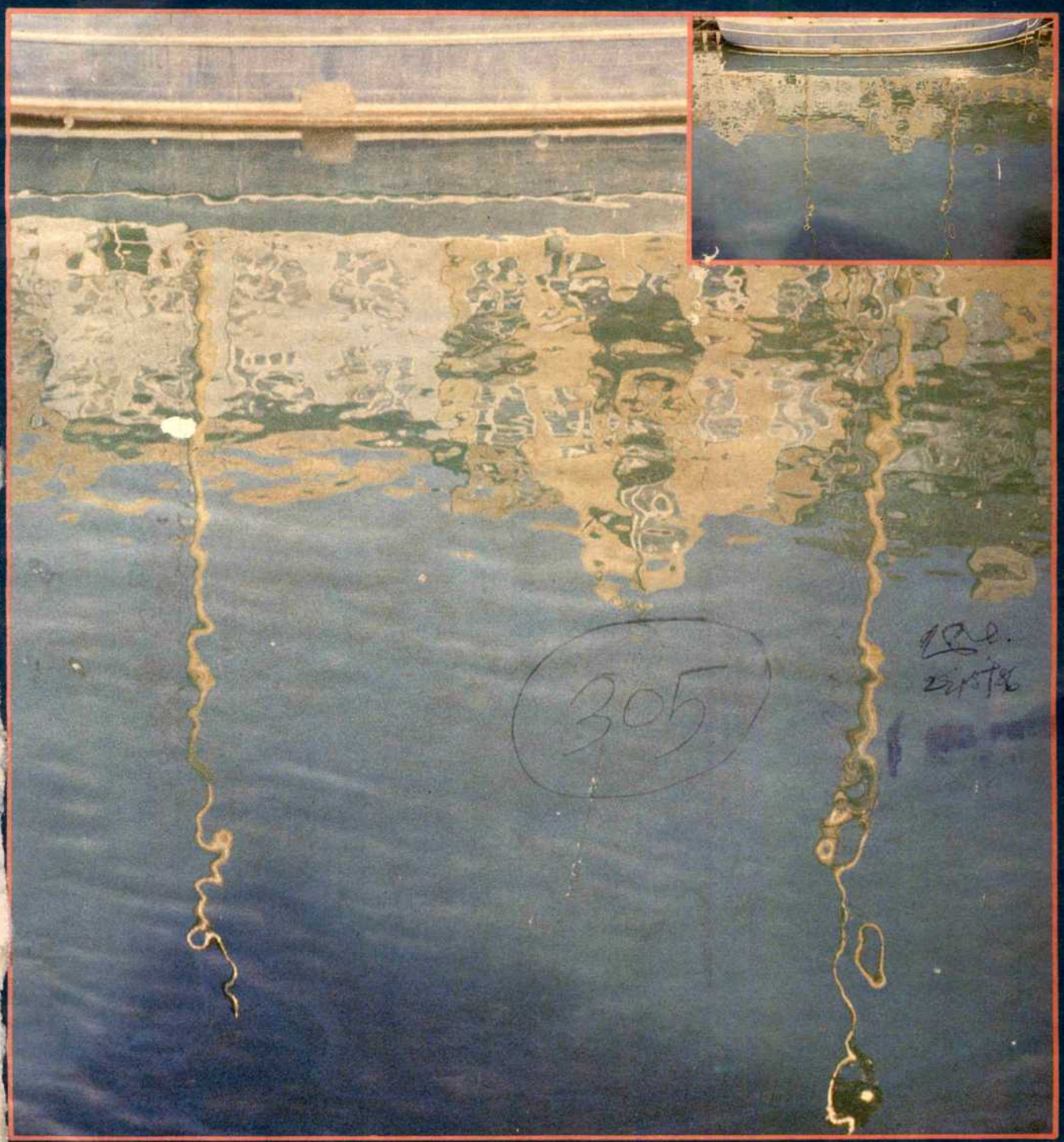
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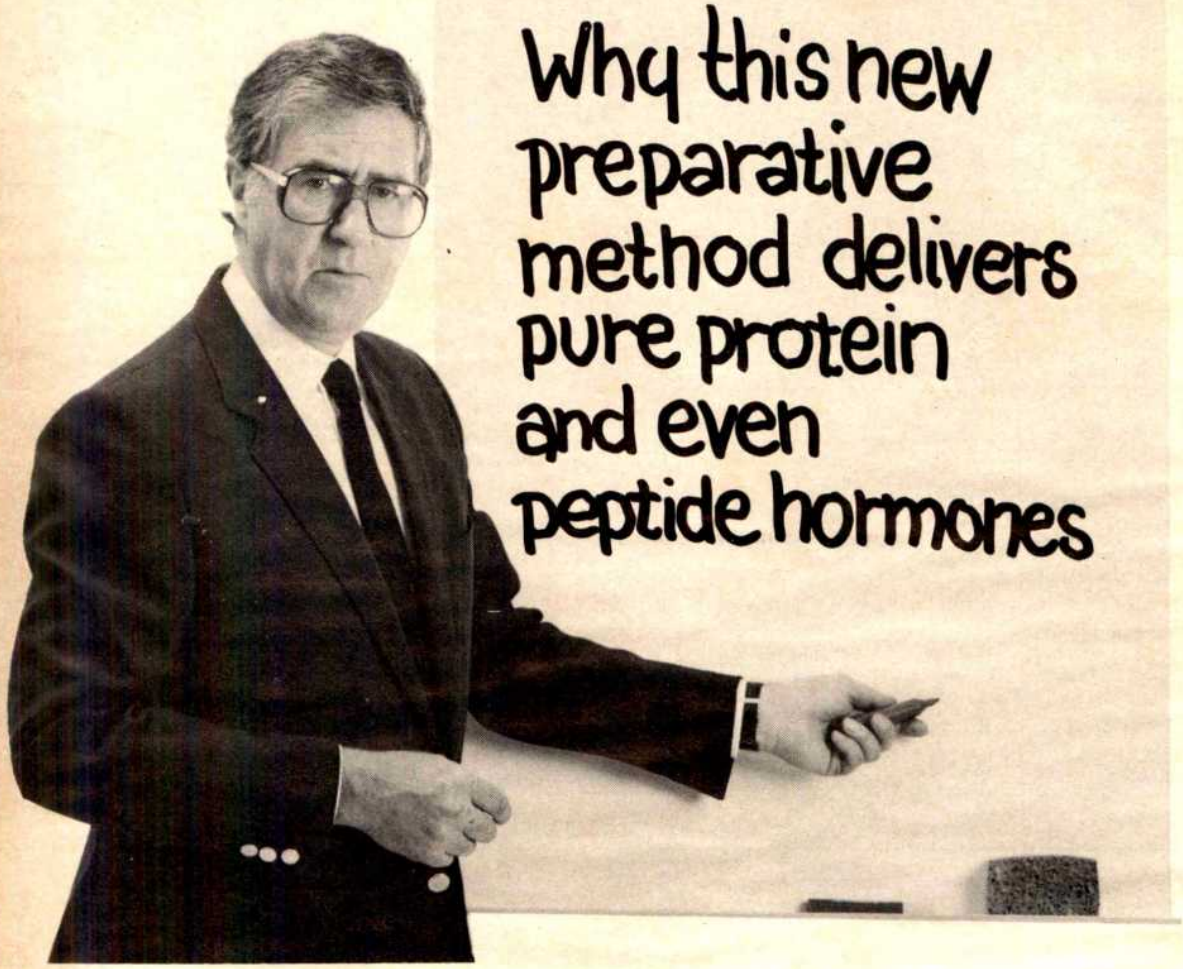
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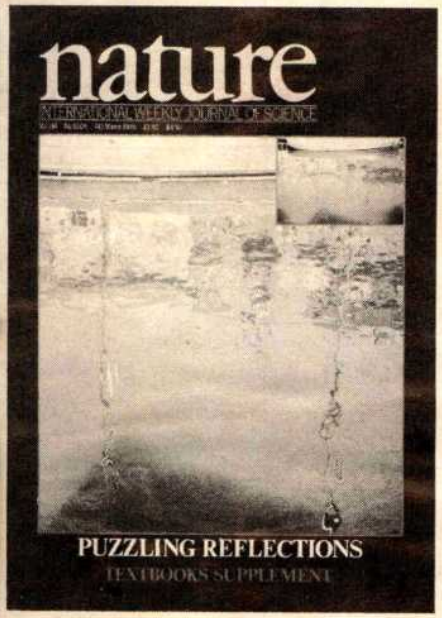
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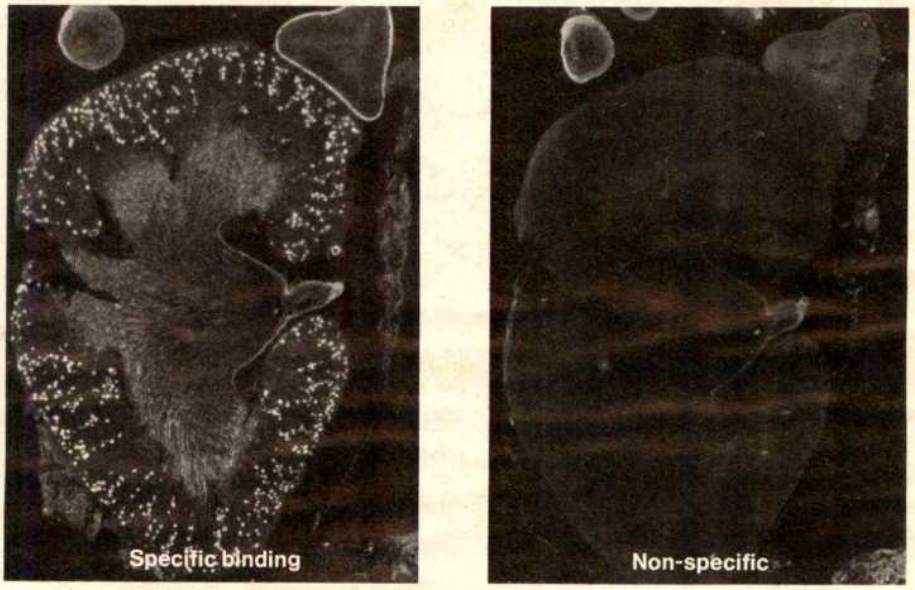
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Amersham's ^{125}I-labelled rat ANP (28-residue sequence) in tissue autoradiography of guinea pig kidney and adrenal. Photographs kindly supplied by Dr P W Mantyh, Center for Ulcer Research and Education, UCLA, USA.

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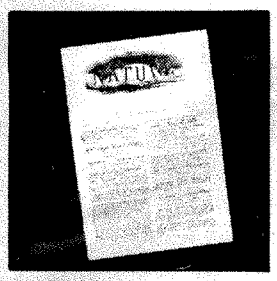
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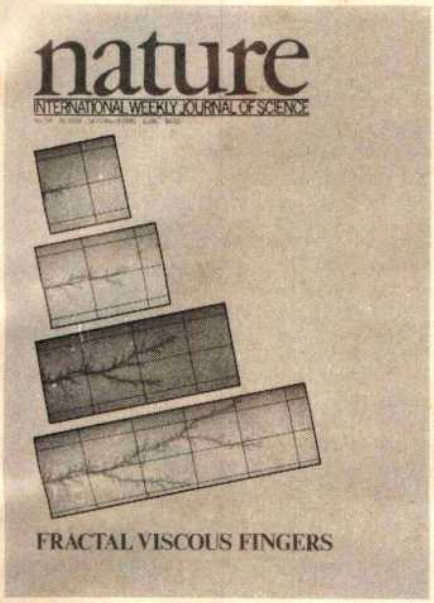
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COVER



FRACTAL VISCOUS FINGERS

When a less viscous fluid like water pushes a more viscous fluid like oil, an instability phenomenon occurs, the 'viscous finger instability', so called because the water forms 'fingers' as it penetrates the oil. It now appears that under certain conditions these highly ramified and irregular patterns are fractals, and can be quantitatively characterized by a number termed the fractal dimensionality (see p.141).

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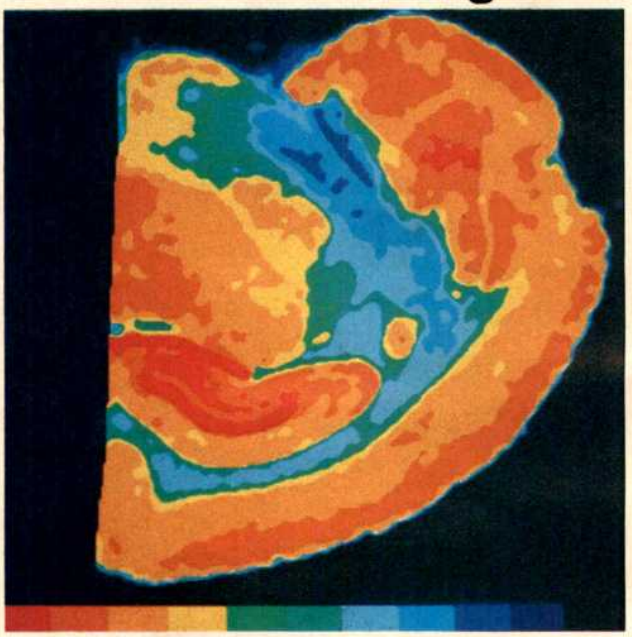
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H[illegible] blood pressure research

Calcium channel ligands



The autoradiographic visualization of Ca^{2+} channels in guinea-pig brain with (−)-[*N-methyl*-^{3}H]desmethoxyverapamil.
Glossmann *et al, Naunyn-Schmiedeberg's Arch. Pharmacol.*, **327,** pp.183–187, 1984.
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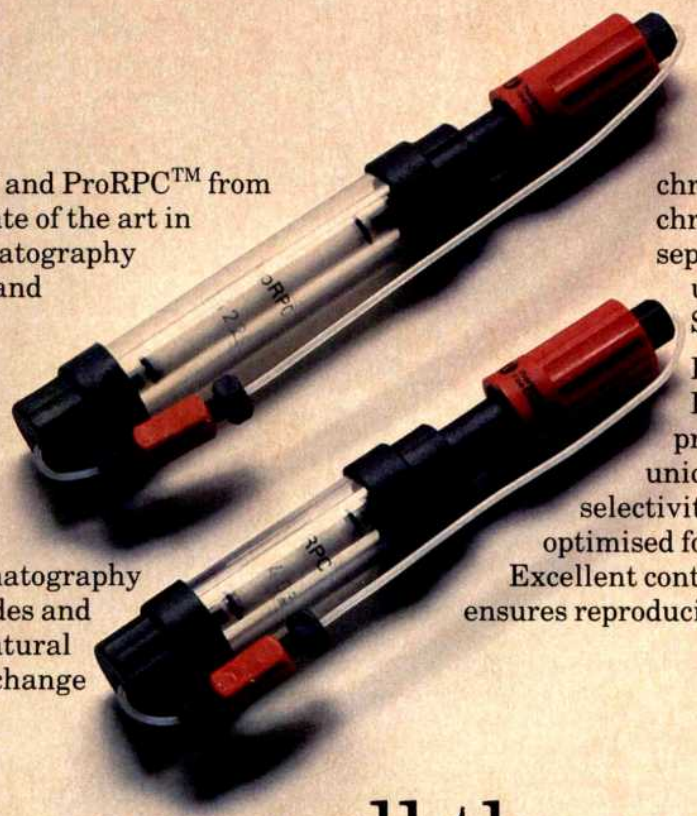
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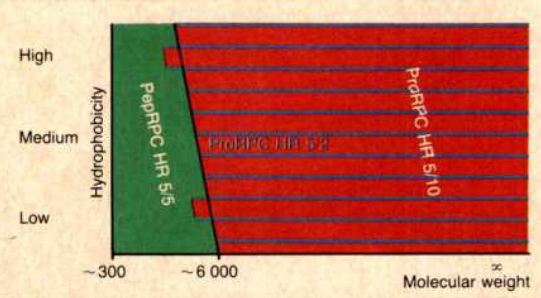
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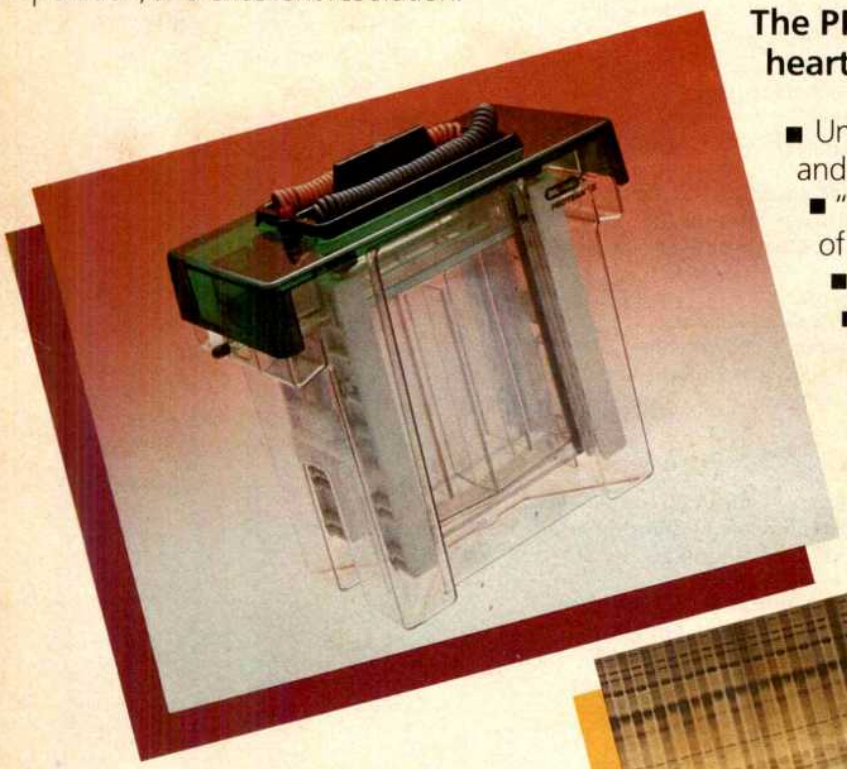
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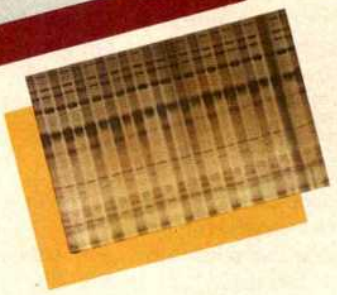
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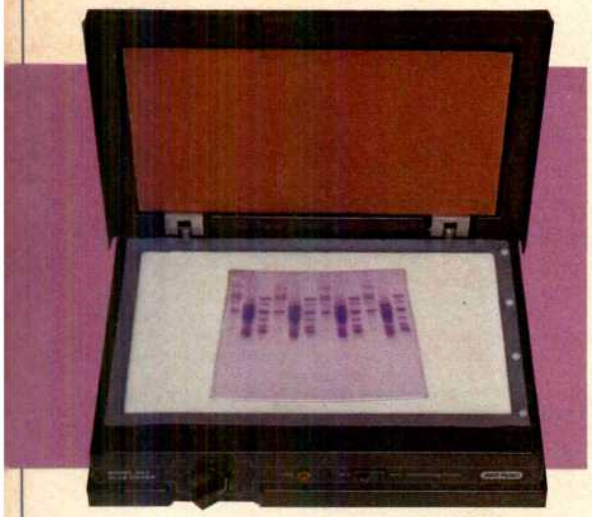


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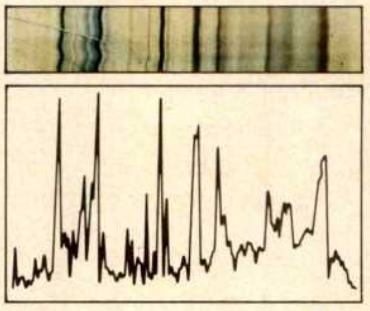


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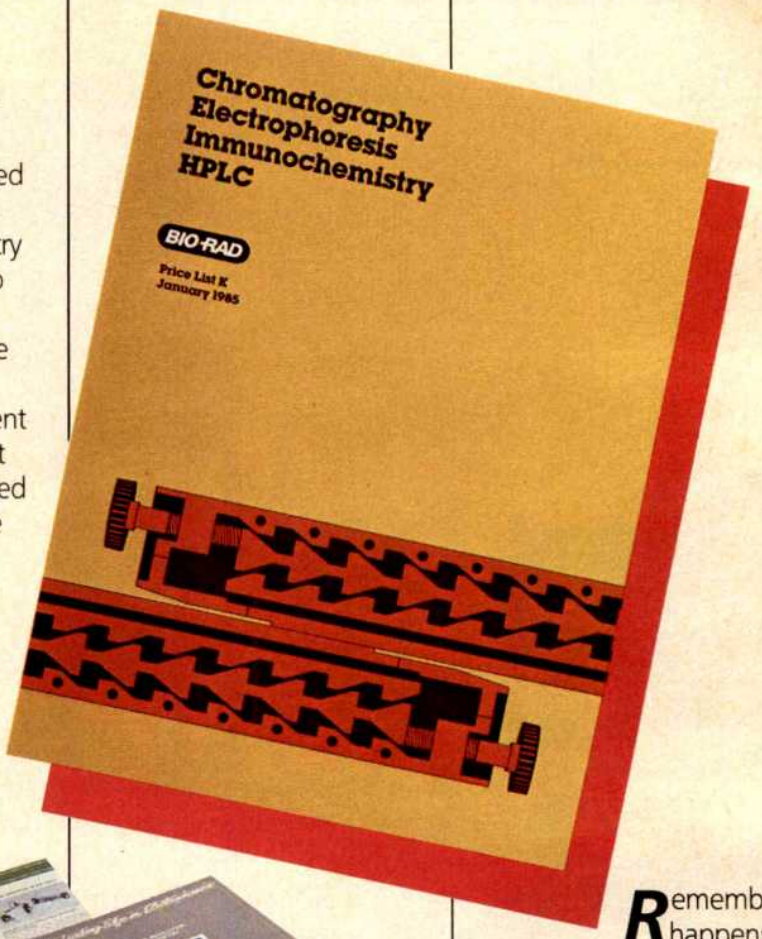
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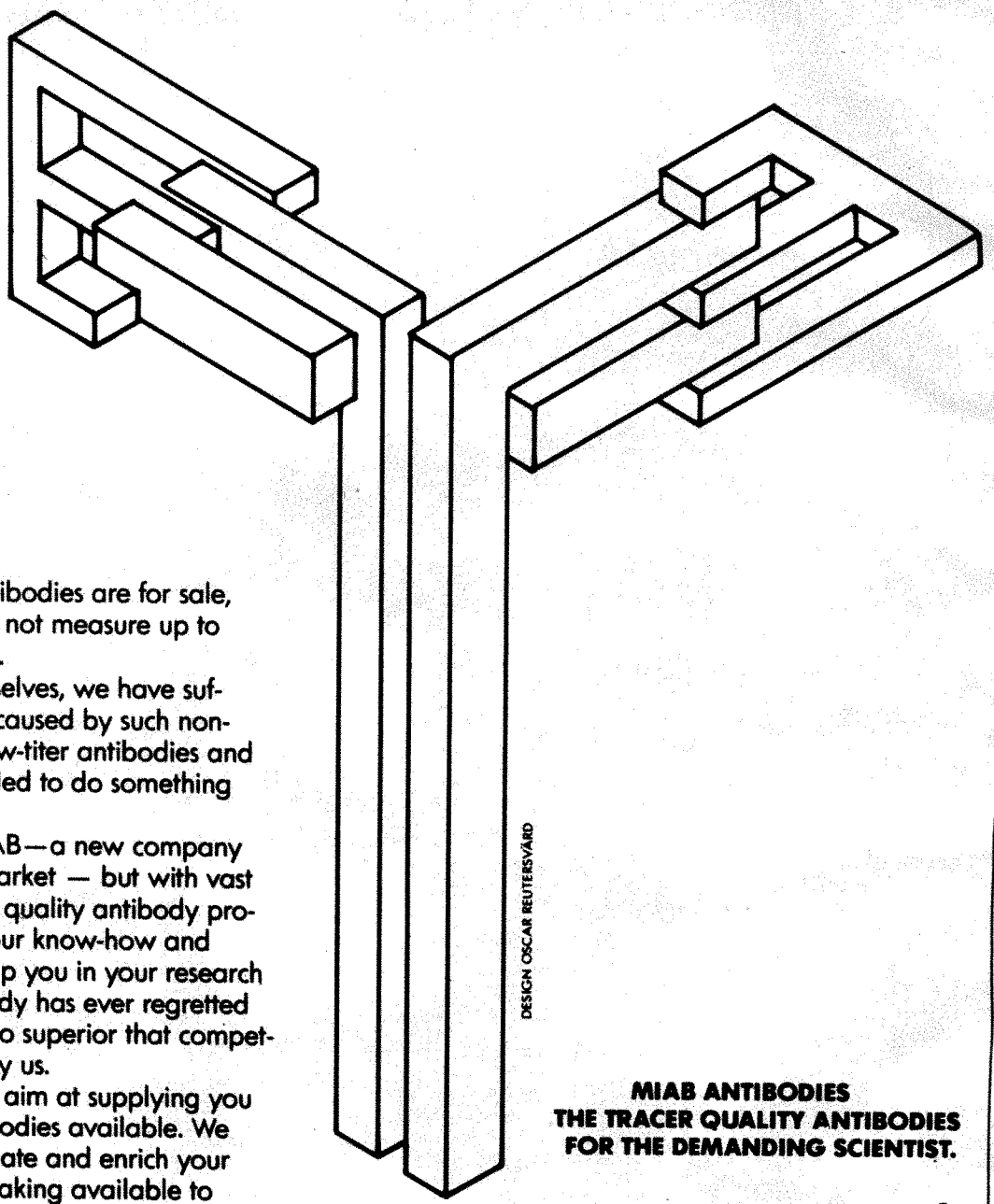
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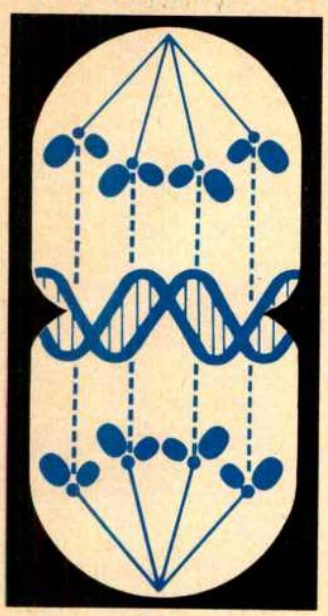
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C. Nüsslein-Volhard (Tübingen)	Early development—*Drosophila*
C. Goodman (Stanford)	Development of invertebrate neural systems
H.R. Horvitz (MIT)	Caenorhabditis

DAY 2: TUESDAY, OCTOBER 8

CELLS

Speakers	*Topics*
M. Davis (Stanford)	T-cell receptors
U. Storb (U. of Washington at Seattle)	B cells
J.M. Bishop (U. of California at San Francisco)	Oncogenes
M. Ptashne (Harvard)	Bacterial development
I. Herskowitz (U. of California at San Francisco)	Yeast development

DAY 3: WEDNESDAY, OCTOBER 9

GENES

Speakers	*Topics*
R. Palmiter (U. of Washington at Seattle)	Transgenic mice
D. Hogness (Stanford)	*Drosophila* homoeotic genes
W. McGinnis (Yale)	Mammalian homoeotic genes
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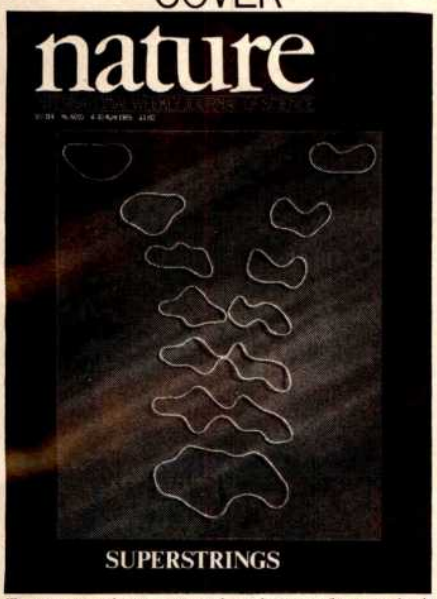
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Two superstrings meet and coalesce to form a single superstring. This is the single, fundamental interaction in the theory of the "heterotic" string, one of a small number of related supersymmetric string theories. These models unite particles and forces — including gravity — in what is almost a "theory of everything". (See p.409). Cosmological consequences of superstrings are described on p.415.

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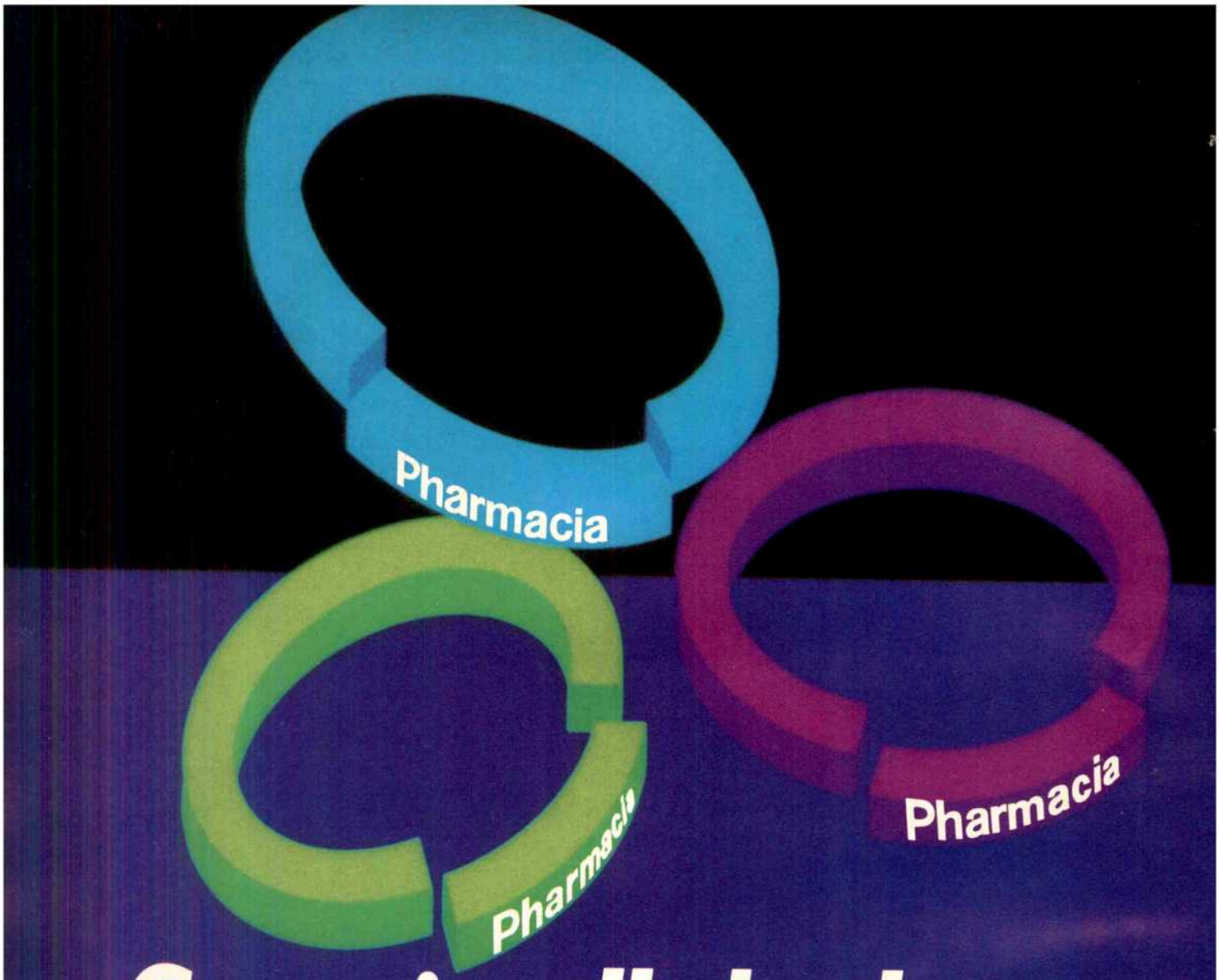
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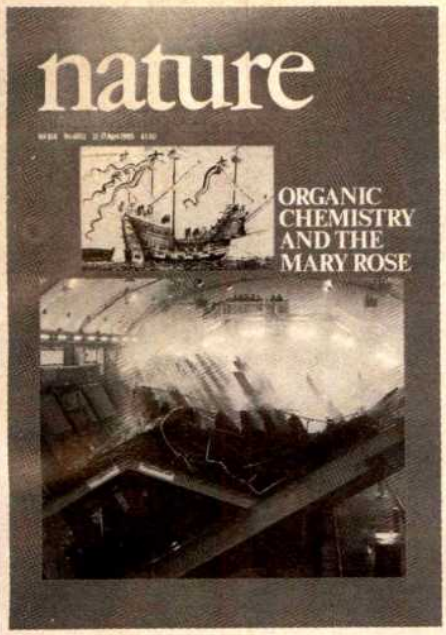
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The *Mary Rose*, from a contemporary watercolour by the civil servant Sir Anthony Anthony (copyright Magdalene College, Cambridge), and the hull as it is today, mounted in a museum cradle at an angle of 60° and showing the viewing platform spanning the dry dock (The Mary Rose Trust). See page 528.

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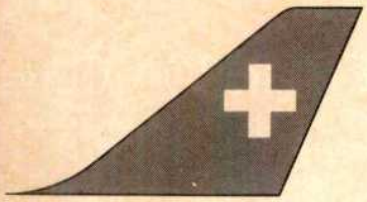
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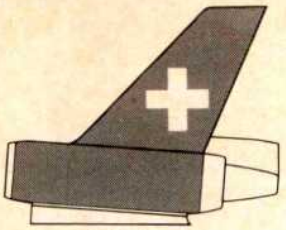
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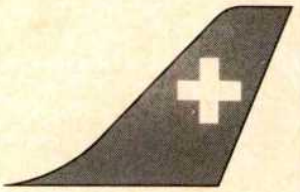
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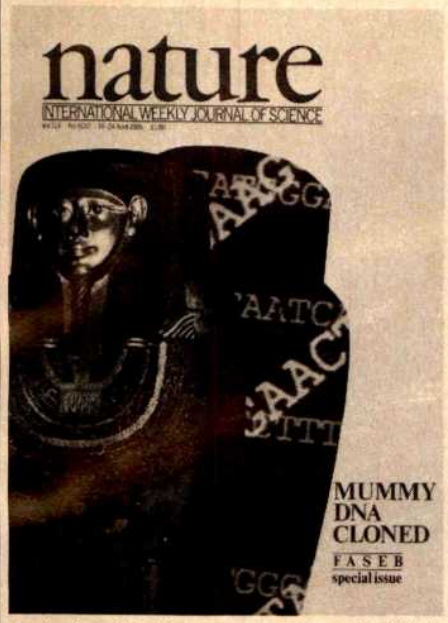
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COVER



An Ancient Egyptian mummy-case from the British Museum, dated at around 200 BC and thought to contain the mummified corpse of a priest of Amun. Tissue from such corpses can be a source of DNA amenable to analysis by modern molecular genetic techniques. On page 644 of this issue the sequence of a fragment of DNA from a 2,400-year-old mummy is reported, and this work is discussed on page 576. (Photos: Ronald Sheriden, Hannu Ukkonen)

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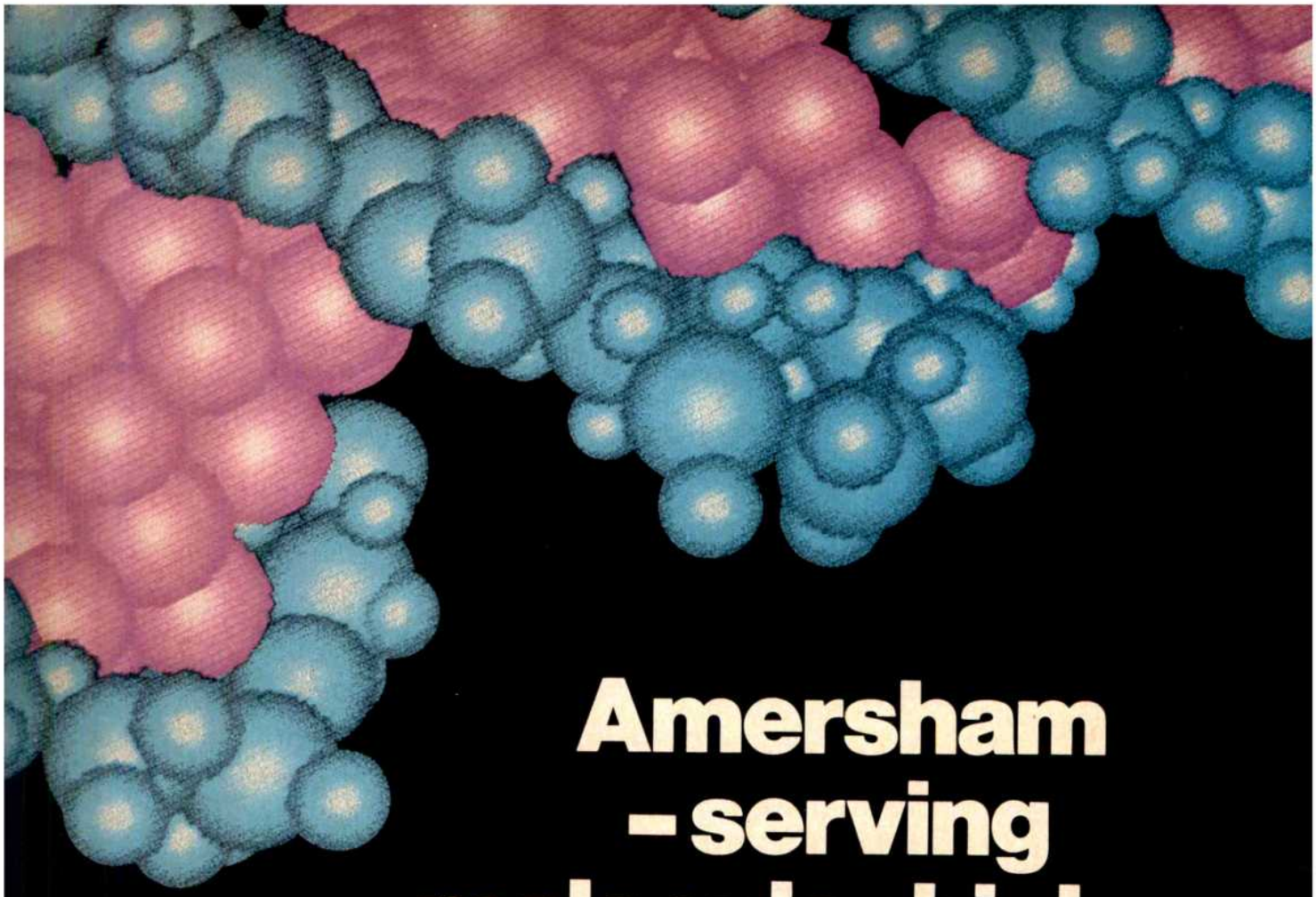
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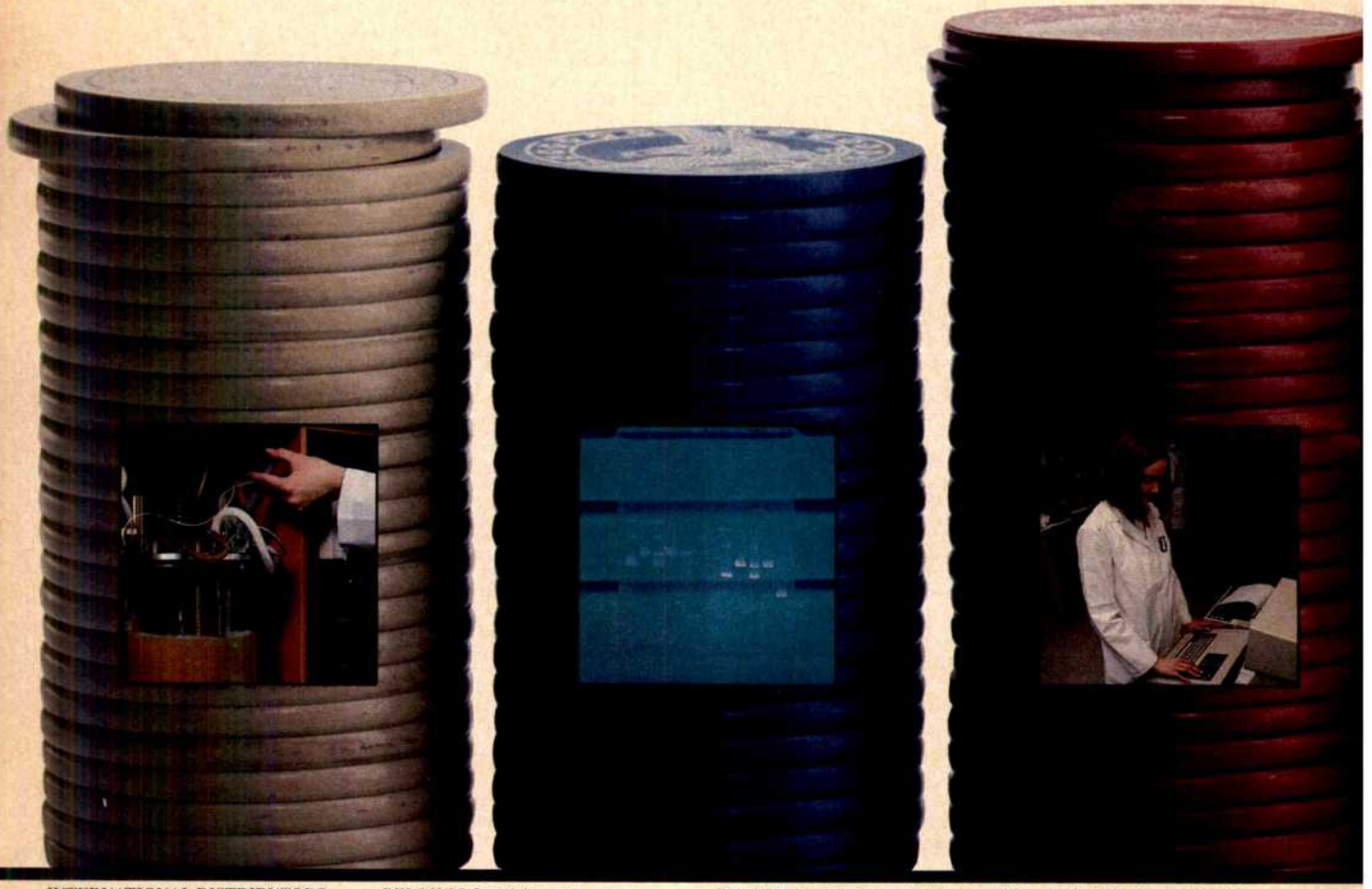
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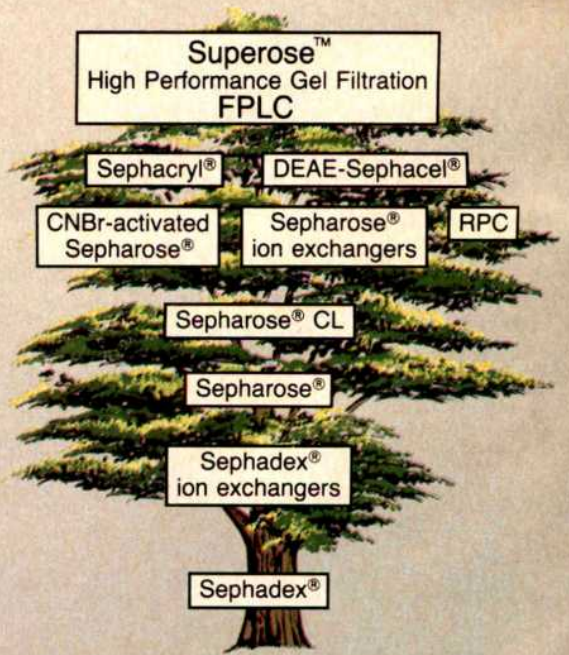
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Changing the objectives of research

The pressures on the British research enterprise, perhaps models of troubles that could arise elsewhere, are made worse by the government's lack of policy. But things may be changing.

WHAT follows is yet another account of the present agonies of the British system of publicly supported research, but that is not a signal to readers working elsewhere (by far the majority) to turn the page in search of something less parochial. For the British difficulty in adapting research and higher education to changing, and generally deteriorating, economic circumstances, is as vivid an illustration as there could be of the difficulties of changing course quickly in those fields. Even where the pace of economic growth is faster, so that the problems of containing spending within tight limits are less acute, there is plenty of evidence that the research enterprise is less flexible than it could be. Those who doubt that proposition should consider the condition of agricultural research in the United States, where attempts to reform the system going back for twenty years have mostly failed.

The British problem has it roots in the 1960s, the decade of relative prosperity when Mr Harold Macmillan, as prime minister, felt it proper to remind his electors that "you've never had it so good". That was the decade in which a threefold expansion of higher education was begun. It was also the decade when the British commitment to the European accelerator laboratory at Geneva was confirmed, and when the present pattern and scale of spending on civil science were determined more or less as they are at present. What else can have been expected of a decade in which the then Mr Harold Wilson (a later prime minister) offered before his election in 1964 a regenerated economy forged in the "white heat" of a technological revolution?

What has happened since is now a matter of record, much of it painful. Since the early 1970s, successive British governments of all kinds have been seeking to shed commitments on the grounds that they had become too expensive. The inquiry now under way under Sir John Kendrew to determine whether Britain should continue as a member of the European centre for high-energy physics (CERN) is not the first of its kind. Since the Rothschild reorganization in 1972, British government doctrine has been that the users of applied research should pay for it, with the result that many of the agents of research have become dependent for large parts of their budget not on the ministries that sponsor them but on a miscellaneous collection of other ministries with no administrative responsibility, either for efficiency or continuity. The present difficulties of the British Geological Survey, one of the largest identifiable components of the Natural Environmental Research Council, have arisen because its customers have become less willing spenders. Now the Agricultural and Food Research Council (AFRC) finds itself in the same case (see p. 8), facing the prospect that an as yet undetermined fraction of £10 million will melt away from its budget next year and probably twice as much in 1987-88. Similarly, British universities (and higher education generally) have for the past four years been made to survive on a budget unrelated to objectives of the enterprise, but instead determined as what the government considered it could afford.

Ignorance

Again in retrospect but also as a matter of immediate concern, the underlying difficulty is that British governments have set about the management of both higher education and civil science without sufficiently serious consideration of what these institution are intended to achieve. The present case of AFRC is a good illustration of how the government's attempt to manage this important part of the research enterprise is marked by what seems remarkably like ignorance of what research can do for an industry. It is not necessary to take sides in the dispute between the research council and the Cabinet Office about the yardsticks by which the scale of agricultural research should be determined to conclude that the system is being mismanaged.

The research council is the largest single agricultural research organization supported with public funds, and gets roughly half of its budget by way of research commissions from the Ministry of Agriculture, Fisheries and Food. But AFRC accounts for rather less than half of all the research supported by public funds in agriculture and related fields. Scotland, in this respect as in too many others, is separate. The ministry itself is responsible for fisheries research, forestry is parcelled out to the Forestry Commission while the ministry carries on its own books research laboratories intended to support veterinary services, among others. In short, there is a ragbag of arrangements for supporting agricultural research, which in normal times offers the advantages of plurality and diversity of research support, but when everything is being made to shrink makes it virtually certain that rationality will take second place after administrative convenience in decisions about which activities should be abandoned. That is one of the strongest reasons why the Scottish agricultural research stations should be united with those elsewhere in Britain, as a House of Commons committee recommended two years ago. The fact that the Scottish system is organized along the lines of the land-grant colleges of the United States (with researchers responsible for the provision of an extension service as well as innovations) should not be allowed to impede the more efficient distribution of effort and resources within this system.

Waste

The present system is also wasteful of such resources as there are. During the present financial year, AFRC will have spent more than £4 million on what is called "restructuring", the standard euphemism for the process of merging institutions and of persuading members of research staffs to take early retirement. Both in the research enterprise and in the British university system, this process has become a serious cause of wasted talents as well as money. As always, the people most willing to retire early are those who can most confidently expect to find something else to do, and who are likely by the same criteria to be most useful to the institutions from which they are displaced. While the theory of this process refers to something called the "managerial interest" by which applications for early retirement are judged, the academic world should by now be aware of the many talented British academic researchers who enjoy a premature pension and an academic salary from some other university system than the British. Is that a sensible way of hoping to turn a country's talent into national wealth? At the very least, the British government should be prepared to moderate the rate at which its research institutions are forced to contract by a sensible calculation of the speed with which that can be accomplished naturally.

Another irony running through recent experience of enforced contraction of the British system is that a government which is constantly complaining that academic research is indifferent to

the national economic interest is nevertheless apparently indifferent to the ways in which beneficial change might be accomplished. Thus the government's forecast of public spending published in January, which foreshadowed the further cuts which now threaten AFRC, boasts in passing that £2 million spent on the genetic improvement of pigs had yielded a benefit of £100 million a year to the pig industry, and that patented innovations in the design of agricultural mowers, themselves the products of a research programme costing £600,000, were yielding an annual income of £750,000 (to the British Treasury) by way of royalties. Sir Keith Joseph, who should know, was saying last week that the British government is soon to announce that such benefits will in future accrue to the organizations responsible — a promise first made in September 1983.

The issue is not merely one of equity, and of providing researchers with incentives to contribute to national wealth, but is also one of proper public administration. For how is it possible for people to make sensible decisions about the contraction of a research programme if they are required only to be concerned with costs? For AFRC, that issue is especially vivid in relation to plant breeding, for the council's excellent Plant Breeding Institute at Cambridge, which costs AFRC roughly £4 million a year, has recently been forced to cut back on staff ironically while the government is brooding on the question whether the National Seed Development Organization, which enjoys the right to market the institute's new varieties and which returned £2 million to the Treasury (half in tax) during its last financial year, should be turned into a private corporation. Why not lump the two institutions together, and let them make their own way in the world?

Core science

These lessons are not directly applicable to what remains the biggest upheaval under way in the British research enterprise, the attempt of the Science and Engineerng Research Council (SERC) to remould its programme of research support so as to release funds for the support of what is called "core science" (physics research for example) while remaining within the limit of a budget shrinking slowly in real terms. Three months of agonizing have identified the options between which the council will have to choose at its meeting later this month. As elsewhere, no decision is likely to avoid diseconomies of one kind (wasting talent) or another (wasting money).

This research council exists exclusively for the support of academic research but has been progressively since the early 1970s caught in the trap that what seemed the sensible provision of facilities for academic research have become institutionalized, representing growing commitments within a budget either stagnant or declining in real value. Astronomy has been one of the council's successes during this period. From a virtually standing start, the British community finds itself with access to new instruments built with SERC participation in Australia, on Hawaii, and now at La Palma in the mid-Atlantic. In Britain, there are three substantial institutions dedicated to the support of these distant telescopes, the Royal Greenwich Observatory, the Royal Observatory, Edinburgh and the Rutherford Appleton Laboratory (which has its finger in many other pies as well). Not all of these can survive the month ahead. The betting seems to be that Britain will pull out of its Australian connection, leaving behind not merely the Anglo-Australian telescope but the excellent Schmidt telescope on the same site, operated for the time being by Britain alone. The obvious difficulty is that funds must somehow be saved, but that it would be too galling, and also uneconomic, to think of pulling away from La Palma, where construction is still under way. But even in the present complicated state of the politics of international observatories and of quotas of observing time, is it entirely beyond the bounds of possibility that a broader international base for construction of the La Palma observatory would keep more facilities alive and accessible? Back in the United Kingdom, it is also clear that one, the other or perhaps both of the observatories must go, but again it is important that neither talent nor money should be wasted for the sake of administrative tidiness. Should not SERC be looking for some way in which the good work these laboratories do (in the development of instruments and the retrieval of data) could be transferred intact to universities, which are not often enough (in Britain) trusted with the execution of projects?

Another of SERC's problems is the clutch of machines that have been built as central facilities for university groups, but which are unlikely to be fully used in the years ahead for lack of funds for the general support of university researchers. There are lasers and synchrotron radiation sources, a neutron spallation source and nuclear structure facility. Again in retrospect, these machines were plainly commissioned too light-heartedly, without a sufficient calculation of whether SERC could afford to support enough customers to keep them fully used. The simple solution is that one or even two of these machines should be abandoned. The more complicated but better solution is that SERC should be enabled to shake free the shackles of chauvinism required of those who spend public money, and make whatever deals may be possible elsewhere in Europe for the continued use of facilities whose virtues, while not necessarily unique, are far from negligible.

SERC's biggest problem in the months ahead is, however, the decision that must be made about the subscription to CERN. The Kendrew committee has not yet reported, and may not until April, but there are only two things it can say — Britain cannot afford to continue to belong to CERN while the cost robs basic science of funds, but equally cannot afford not to belong. So there will be a period of further introspection, the issue will fall to be resolved by the government, which may or may not provide the extra cash that would be needed to square Kendrew's circle. But that will be a shabby way in which to deal with a serious problem — and what is in effect a treaty obligation. Walking out of CERN would not merely confirm mainland suspicions that Britain is a reluctant European but would damage an important laboratory at a particularly interesting time in its development. What the government (and SERC in its own interests) might reasonably expect, however, is that the future development at CERN, beyond the machine now planned (called LEP, an electron-positron collider), should be undertaken on a different financial basis, perhaps involving only the enthusiasts and the very rich. That way, the scale of SERC's dilemma would be limited in time.

What chance is there that the British government, hard-faced as it has been in the past five years, will listen to this special pleading? On past form, not much. But there is now at least a chance that the tide may be on the turn. After setting its face against the natural growth of student numbers at British universities, the government is now about to announce a planned increase, sufficient to yield about 1,000 graduates a year in science and engineering. Similarly, it seems the government has been more flexible that expected over the extra cost of continued membership of the European Space Agency. In each case the budgets of other ministries have been raided so as to provide urgently needed funds for education and science, necessary for carrying through policies on which the government has decided. Is it possible that a government whose reputation is for inflexibility has begun to appreciate that policies and not book-keeping should dictate the pattern of its spending?

That must be the hope. The British government's case for containing public spending within strict limits is strong. The need will continue for years to come. There may also be something in the government's constant complaint that the British investment in research has not yet been as productive of profitable innovation as might have been expected. But during the past five years, the management of the system has taken the form of publishing once every year figures showing what costs will be allowed and then grumbling from the sidelines. As anybody could have predicted, and as many did, the result within the system has been diseconomy. Nobody will suggest that governments, or even civil servants, should take on themselves the direction of research. But if they spend upwards of £500 million a year on these activities, they cannot walk away from the need, first, to help define their expectations and, second, to ensure that the resources are available. □

British higher education

Modest increase planned in student numbers

THE British government plans to announce next week an increase in the numbers of students following science and engineering courses at universities. This decision is the outcome of government alarm during the past two years at the shortages of trained people free for recruitment to British industry. But to many academics, the development will seem like the light at the end of a four-year tunnel.

The intention is that between £40 and £50 million should be spent over the three years beginning in September on the provision of extra places in science and engineering. Some of the money will come from the budget of the University Grants Committee, but the novelty of the plan (due to be made final at a meeting on Wednesday, 6 March) is that other government departments will be contributing. The Department of Trade and Industry and the Department of Energy are among those that have agreed to contribute from resources they would otherwise have spent on their own account.

In one respect, the new development is an outcome of the plan, widely canvassed a year ago, for organizing a "switch" within British higher education from the arts and humanities to science and engineering. That was frustrated by the unwillingness of departments other than the Department of Education and Science to pay up. One feature of what is now intended is that the increase of science places (smaller than first planned) will not be offset by an actual reduction of the places available in the arts and humanities.

The scheme about to be launched will apply only to the universities. Although, in early discussions, the possibility was canvassed that polytechnics might also be included, representations by employers to the Department of Trade and Industry seem to have convinced the government that university graduates are most needed.

One of the questions to be decided on 6 March is whether the Open University and Cranfield Institute of Technology, which are supported directly by the Department of Education and Science, should participate.

The intention is that formal applications for a share of the newly available resources should be invited from universities during April, and that funds should be available both for supporting courses and for modest building projects. The urgency of the timetable the government has set for itself is dictated by the need to get things started at the beginning of the next academic year.

Among those participating in the planning of this new departure are some who fear that students with school-leaving qualifications of the kind required by most university engineering courses may not be available in sufficient numbers in the years immediately ahead.

Among academics, the chief import of the new development will seem to be the evidence it provides of a new flexibility in the British government's management of resources. Some think that the partial defeat last year of Sir Keith Joseph's attempt at redistribution within his own budget may have persuaded ministers that the system had become inflexible. □

Space budget fixed

A FEW weeks ago, the UK Science and Engineering Research Council (SERC) was beside itself with worry over how to pay an increased mandatory subscription to the European Space Agency (ESA), to pay for ESA basic science. But now, in an important decision of principle by the British government, it appears that SERC is to be taken off the hook.

The trouble began in January when European space ministers agreed a 10-year programme for ESA. The programme included a three-per-cent annual increase (eight per cent in UK cash terms) for the next five years in the ESA science budget. The straitened SERC, which belongs to the budget of the Department of Education and Science (DES), could not pay. Yet the subscription was due if the Department of Trade and Industry (DTI) was to participate, as it wished to do, in ESA's plan to join the US space station. The usual Treasury procedures did not allow DES to claim the increase from DTI.

Deadlock seemed likely. But now, according to DES, "SERC's additional costs are likely to be shared among various departments" — a statement that marks a fundamental breaking of interdepartmental barriers.

Meanwhile, discussions over a possible British "space centre" appear to be faltering over the location and powers of the centre. Several sites are being considered. Another less auspicious possibility is that the "centre" would be no more than a mere coordinating committee. Robert Walgate

US supercomputers

Four research centres named

Washington

THE National Science Foundation (NSF) has finally put its money where its governing board's mouth has been and awarded a total of $200 million to set up supercomputer centres. They will be at the University of California at San Diego; Cornell University, in Ithaca, New York; the University of Illinois, Champaign-Urbana; and Princeton, New Jersey, near the campus of Princeton University.

Location	Machine	Director
San Diego	Cray XMP	Sidney Karin
Champaign-Urbana	Cray XMP	Larry Smarr
Ithaca	IBM 3084 QX/ FPS 164s and 264s	Kenneth Wilson
Princeton	CDC Cyber 205 (after 1986, ETA-10)	Steven Orszag

NSF is to provide each centre with between $7 million and $13 million a year for the next five years for the purchase and operation of the equipment. Users will not have to pay for computer time, which will be allocated both by NSF directly — in the case of requests for large blocks of time submitted as part of NSF grant applications — and by research committees at each centre. NSF officials expect a roughly 50-50 split between these two.

Two years ago, a working group of the National Science Board, NSF's governing body, called for a $200 million investment in ten supercomputer centres on the grounds that "important science" was already being neglected in the United States for lack of supercomputer capacity. The recommendation was received more warmly by Congress than by the administration's budget-writers. So far, NSF's official response has been to buy time on existing supercomputers, underwriting the equipment, such as terminals and communications networks, that academic researchers have needed in order to gain access to those machines.

Under the contracts announced last week, the states in which the four centres are to be located will contribute an average of about $20 million each. The host institutions will contribute about $10 million each, mainly in land and buildings, while computer manufacturers will supply an additional $50-$70 million, mainly by giving equipment or providing it at reduced prices.

According to NSF, the four host institutions, together with 28 other universities that will be affiliated with the San Diego and Princeton centres through two consortia, will be able to "influence policies and procedures" of the centres but will have to compete on an equal footing with all users for time. The centres are expected to begin operating in late 1985 or early 1986. **Stephen Budiansky**

US-Australian defence

Hawke fights on second front

Canberra

THE Australian Labor government seems to have successfully contained the damage that might have been done by last month's visit to Washington by Mr R. J. (Bob) Hawke, the Prime Minister. The issue is Mr Hawke's widely reported reversal of an earlier decision to provide logistical support for two US MX missile tests to be held this year in the Tasman Sea, off Australia's east coast. The government had feared that internal faction fights would cause trouble. Stiffer tests of consensus are to come, however. Mr Hawke has come under continuing pressure from the United States to strengthen US-Australian bilateral defence agreements and to promote the growing isolation of neighbouring New Zealand, following that country's refusal of port facilities to a nuclear-capable US warship earlier this year. The three countries are bound together militarily by the ANZUS treaty. On 4 March, however, Mr Hawke announced that the next ANZUS council meeting scheduled for July had been indefinitely postponed. Strictly speaking, under the treaty the three countries are obliged only to "consult together" in time of threat. But in practice ANZUS has been the formal basis for the US security guarantee to Australia and New Zealand for thirty-three years. Cancellation of the council meeting signals its likely imminent demise.

Mr Hawke's difficulties began immediately before his departure from Australia on 2 February for meetings in the United States with President Reagan and Secretary of State George Schultz, when he was reported to have agreed, without consulting his full cabinet or caucus, to provide facilities for US monitoring of the MX splashdown area.

The agreement would have fulfilled an undertaking by former prime minister Mr Malcolm Fraser, and would have made it possible to test the accuracy of the four-stage missile over its full range of 14,000 km, rather than over the shorter Vandenberg-Kwajalein range. With ten 350-kiloton warheads, the accuracy of the MX over a distance of 7,000 km is approximately 90 metres using NAVSTAR guidance.

Under pressure from the Labor Party's left wing, Mr Hawke cabled from Brussels on 6 February (the day before he was due to meet President Reagan) his decision to withdraw from the tests. The switch was made to look even more humiliating by Mr Hawke's exhortation three weeks earlier to New Zealand Prime Minister David Lange to rescind his decision to deny the use of port facilities to the destroyer USS *Buchanan* if the ship were to be carrying nuclear weapons, an item of information routinely refused by the US Navy.

Despite the MX fiasco, Mr Hawke has been let off much more lightly by Washington than has Mr Lange. There appear to be several reasons for this. One is a desire to counter the growing influence of the Australian government's left wing, another to send a message to wavering NATO allies emboldened by Mr Lange's



actions, and a third the importance the United States attaches to its Communications Command Control and Intelligence (C^3I) bases on Australian soil.

Information about these installations is scarce and more likely to be uncovered in US publications or under the US Freedom of Information Act than through Australian government channels, but there are thought to be more than two dozen of them.

The continued presence of these stations in Australia has become a contentious issue. Critics of the facilities at Pine Gap and Nurrungar (see below) in particular charge that whatever their value when they were commissioned, during the "deterrence" years, they are now (or could become) part of a first-strike "counter-force" system and hence nuclear targets. This strikes at the heart of Mr Hawke's embattled strategy for peace and disarmament, a major plank of government policy.

The New Zealand government is soon to become even more isolated militarily after Mr Hawke's announcement last week that New Zealand will in future be denied the usually generous US component of the intelligence "product" formerly shared among the United States, United Kingdom, Australia and New Zealand and Canada under the 1947 UKUSA secret treaty. Practical problems of intelligence separation have arisen for the Australian government because intelligence from the "joint facilities" combines information from shared sources as well as that gathered solely by Australia. This is compounded by the presence (on secondment) of New Zealand intelligence officers in several sensitive Australian installations. Mr Hawke must now deal with the looming policy problems of south-west Pacific regional defence, at the same time avoiding the impression that Australia is dumping a small idealistic neighbour at the behest of the United States. **Jeffrey Sellar**

US defence facilities in Australia

OFFICIALLY referred to as "joint US-Australian facilities, the most important US defence installations in Australia are, according to Dr Desmond Ball of the Australian National University, the following.

Naval Communications Station *Harold E. Holt*, North West Cape.

Operational since 1967, this station maintains reliable communications with the nuclear-powered ballistic missile submarines of the US fleet patrolling the Pacific Ocean. The VLF (Very Low Frequency) facility is the largest and most powerful in the US worldwide communications system. North West Cape also has an array of high-frequency transmitters used for US military operations in the Indian and Western Pacific Oceans, as well as a ground station for the US Defense Satellite Communications Systems (DSCS).

Joint Defense Space Research Facility, Alice Springs (known as Pine Gap).

Operational since 1969, the facility is 19 km south-west of Alice Springs and consists of seven large radomes and a large computer complex. Pine Gap was originally established as part of Project Rhyolite, which involves a small number of very large antenna-carrying signals-intelligence (SIGINT) satellites in geostationary orbit. The facility monitors a wide spectrum of Soviet and Chinese military communications and radar transmissions, allowing the mapping of the extensive Soviet early-warning and air defence networks, as well as telemetry data transmitted during Soviet ballistic missile tests. This is the principal means of monitoring Soviet missile developments, and hence Soviet compliance with the strategic arms agreements.

Joint Defense Space Communications Station, Woomera (known as Nurrungar):

This is within the Woomera restricted area, 480 km north-west of Adelaide, and with Buckley, Colorado, is one of the two ground stations for the US satellite early-warning system. Nurrungar provides a real-time link between the North American Air Defense Command (NORAD), Strategic Air Command (SAC), the national military command system and the satellite early-warning system. Data are derived from infrared, charged particle and radiation sensors aboard the geostationary satellites of Program 647 of the Defense Support Program (DSP), which detect missiles shortly after lift-off. **Jeffrey Sellar**

Polish universities

Government legislation unwanted

Warsaw

THE "public debate" over Poland's 1982 Higher Education Act is becoming, more and more, an open confrontation between the universities and the government. A referendum last week among academic staff at Warsaw Universitiy gave an overwhelming vote of "no" to the proposed changes. (More that 75 per cent were against the reduction of the size of the senate, and more than 80 per cent against all other reforms.) A similar referendum among the students, now in progress, seems likely to give even more conclusive results. Yet the votes are unlikely to make any real difference. The government, it is believed, will simply push through the changes.

According to those who oppose the reforms, the changes would effectively annul the academic autonomy gained in the Solidarity period. Rectors and deans, at present elected, would be nominated by the Minister of Science and Higher Education, who would also have wide powers to suspend or dismiss staff, students and academic bodies. Political as well as professorial considerations would be taken into account in hiring university staff, and the "students' self-government committees" would be replaced by new bodies, based on the party-linked youth organizations.

The government case is that, since the state supports the universities, the state has a right to decide what goes on in them, and, in particular, to ensure that they are not used for anti-socialist purposes. The proposed changes in the act would therefore stress the explicitly "socialist" nature of Polish education, and wide disciplinary powers in the case of alleged "anti-socialist action" by staff or students. (The definition of such action will, it appears, be wide according to sources in Warsaw Polytechnic. The rector of that institution is under pressure from the ministry to discipline students who carried the polytechnic's banner in the funeral procession of the murdered pro-Solidarity priest Father Jerzy Popieluszko, last autumn.) All that is happening, one ministry spokesman tried to explain, is that the Committee for Social and Political Affairs if trying to plug a few loopholes in the 1982 act, which have been used by opponents to destabilize the universities.

The academics, on the other hand, maintain that nothing is more likely to destabilize the universities than constantly changing the law.

The Main Council of Higher Education (which consists of one delegate from every university and higher college in Poland, with the exception of the Catholic University of Lublin) has repeatedly made the point that the present act should be allowed to run for the term of office of the rectors elected in 1984 (until 1987), before any changes are discussed.

One scholar formerly active in the unofficial Society of Academic Courses, the "Flying University" of the late 1970s, went so far as to say that what is at stake is not the election versus appointment of rectors, nor the existence of the "student self-government committees", nor even the right of the minister to suspend or dismiss recalcitrant staff or students. "What we are fighting for", he said, "is to ensure that the government cannot simply change the law every time it wants to interfere in the universities". **Vera Rich**

UK research

Joseph warns of trouble ahead

THE whole of the British research establishment spent two hours at the Savoy Hotel last week to hear Sir Keith Joseph, the Secretary of State for Education and Science, deliver a speech whose preparation cannot have taken nearly as much time as that needed to feed the multitude. He was speaking at the annual luncheon of the Parliamentary and Scientific Committee.

Sir Keith had three points to make, of which the clearest was that the scientific community should address itself to the "moral dilemmas" occasioned by new developments in research. He said that the parliamentary bill introduced by Mr Enoch Powell MP, which would ban the use of human embryos for any purpose other than the treatment of infertility, is only "an introduction" to the troubles that lie ahead.

On support for research by the government, Sir Keith said that his partially successful attempt to transfer funds from student maintenance grants to research support had broken new ground by uniting all sections of his party in opposition. He went on to chide scientists in his audience because, he said, among the "uncounted" letters of protest about student grants, there had so far been found none — "not one" — applauding his attempt to increase research support.

Borrowing from C.P. Snow, Sir Keith also wrung his hands last week over what he called "the two cultures" of "science and trade". He complained of the "suicidal snobbery of academic attitudes" towards trade, arguing that the stagnation of British support for research is a consequence of poor economic growth in recent years.

But Sir Keith said there are now "harbingers of improvement" to be found both in the rate at which new enterprises are being created in Britain and in developments such as that of the complex of science-based industries recently grown up around Cambridge. **John Maddox**

British AIDS

New cases cause alarm

SINCE the summer of 1982, 132 cases of acquired immune deficiency syndrome (AIDS) have been diagnosed in Britain, 58 of which have proved fatal. But not until last month was public anxiety about the disease fanned, following the publicity given to the death of a prison chaplain.

The Department of Health has now set up an expert advisory group representing science and medicine and public health services. The disease has not been made notifiable, although patients with AIDS are reported to the Communicable Disease Surveillance Centre by their doctors. Government policy is fivefold:

- Guidelines for all health professionals.
- Health Education Council leaflets to promote public awareness of the disease particularly among "at risk" groups (male homosexuals and intravenous drug abusers).
- Safeguarding of recipients of blood and blood products. Blood transfusion centres are distributing widely a leaflet *AIDS: Important New Advice for Blood Donors*; the British AIDS charity (the Terence Higgins Trust) and the Blood Transfusion Service are asking "at risk" people not to give blood.
- Development of tests to screen blood donations for the AIDS virus antibody.
- Speeding-up of the programme to produce heat-treated factor VIII (the blood-clotting factor) used to treat haemophiliacs. The Haemophilia Society is selling a comprehensive booklet about AIDS.

The government's financial commitment to AIDS research consists of £158,000 from the Medical Research Council (MRC) over three years (from October 1984), supplemented by £15,000 a year from the health department. MRC has also committed £27,000 for two years (with £7,000 a year extra from the health department) for research on the relationship of AIDS to patients with blood disorders. The US AIDS research budget is $86 million a year (see *Nature* 28 February, p.725).

The AIDS virus has been isolated independently in three different laboratories and has been variously named the lymphadenopathy-associated virus (LAV), human T-cell leukaemia/lymphoma virus (HTLV) type III or AIDS-associated retrovirus (ARV). It is now recognized that all three are variants of the same virus (see *Nature* **313**, 636 and 743; 1985) whose nucleotide sequences differ by 1 per cent and whose genetic organization is identical. The AIDS virus is thought not to be a member of the HTLV family of retroviruses, but to represent a new class.

According to Department of Health figures, 90 per cent of deaths due to AIDS in Britain have been male homosexuals of average age 36. **Maxine Clarke**

Genetic manipulation

Rifkin battle lost, war undecided

Washington

A PANEL of the US Court of Appeals has ruled invalid a lower-court injunction forbidding the National Institutes of Health (NIH) to approve any deliberate-release experiments (see *Nature* **312**, 689; 1984). But the Lindow experiment on frost-resisting bacteria is still held up pending approval of a formal document assessing its environmental risks.

The lower-court injunction, granted to anti-genetic-engineering activist Jeremy Rifkin, had ordered NIH's Recombinant DNA Advisory Committee (RAC) to prepare a lengthy "environmental impact statement" before considering any other proposals for field trials of recombinant organisms. NIH officials said that preparing such a document would have taken a year and would itself have been subject to further legal challenges.

The Lindow experiment, in which genetically-modified bacteria would be sprayed on potato plants to protect against frost damage, was to be the first in which bacteria containing recombinant DNA were to be released to the open environment. The experiment was approved by RAC in June 1983; in August of that year, Rifkin sued in federal court to halt it.

Although the appeals court has removed the biggest threat that Rifkin held over RAC — the prospect of tying up all deliberate release experiments in years of legal manoeuvring — it left no doubt that NIH did not follow the law in approving the Lindow experiment without first completing a formal environmental assessment. The court said that environmental assessments would likewise be required for each deliberate-release experiment that comes before RAC.

As for the Lindow experiment, it is now apparently up to the lower court in the person of District Court Judge John Sirica to decide whether the environmental assessment that the RAC staff has in fact just completed on the experiment is adequate. Rifkin's lawyer has raised the possibility of challenging that point. Rifkin himself has said on several occasions that he does not believe that there is at present sufficient knowledge fully to assess the environmental risks posed by the deliberate release of recombinant organisms.

The court's ruling also opens the way for routine legal challenges of every deliberate-release experiment that comes before RAC on the grounds that the environmental assessment is inadequate.

NIH are meanwhile taking steps to get out of the business altogether. The Environmental Protection Agency (EPA) intends to regulate all field trials of "microbial pesticides" that have been genetically altered or manipulated; under new EPA rules, the agency would have to be given 90 days' notice of any such test. (Previously, EPA regulations applied to field trials of pesticides only when the test plot exceeded 10 acres.)

EPA is also working on regulations that would apply to field trials of other recombinant organisms as well. At the next RAC meeting, scheduled for 3 May, the committee is expected to approve a proposal that would allow researchers subject to the RAC guidelines (currently any institution that receives federal funds for recombinant DNA activities) to go ahead with deliberate release experiments so long as they have approval from another federal agency; RAC approval would no longer always be required.

RAC has not received any new proposals for deliberate-release experiments from federally-funded institutions since the Lindow approval went to the courts. Two private companies, however, Advanced Genetic Sciences (which proposed an experiment virtually identical to Lindow's) and Cetus Madison (which proposed a plant experiment), voluntarily submitted proposals last year; RAC approved them, but the NIH director, James Wyngaarden, decided that even though the injunction did not apply to private companies (which are not legally bound by the RAC guidelines), there should be no double standards, so he is holding up approval until environmental assessments are prepared for them. Advanced Genetic Sciences has since withdrawn its proposals and has decided to deal only with EPA; Cetus Madison has been told that if it still wants its proposal considered by NIH, it should submit a draft environmental assessment.

Stephen Budiansky

Court wishes plague on both sides

THE following are extracts from the opinion of the United States Court of Appeals in the case in which NIH appealed from a ruling of the lower court awarding an injunction on deliberate-release experiments to the Foundation on Economic Trends, Mr Jeremy Rifkin's organization.

Judge Wright

This appeal presents an important question at the dawn of the genetic engineering age: what is the appropriate level of environmental review required of the National Institutes of Health (NIH) before it approves the deliberate release of genetically engineered, recombinant-DNA-containing organisms into the open environment?

We emphatically agree with the district court's conclusion that NIH has not yet displayed the rigorous attention to environmental concerns demanded by law. We therefore affirm the district court's injunction prohibiting the University of California deliberate-release experiment until an appropriate environmental assessment is completed. We also share the district court's view that NIH should give greater consideration to the broad environmental issues attendant on all deliberate release of organisms containing recombinant DNA, and to its own responsibility for approving these deliberate-release experiments. We find, however, that the part of the injunction enjoining NIH from approving all other deliberate-release experiments is, at this juncture, overly broad, and we therefore vacate the part of the injunction that prohibits NIH approval of those experiments. We emphasize, however, that if NIH fails to give appropriate environmental consideration to any other experiment, as it has failed to do with the University of California experiment, injunctive relief would be clearly proper.

Broad claims are made about both the potential benefits and the potential hazards of genetically engineered organisms. Use of recombinant DNA may lead to welcome advances in such areas as food production and disease control. At the same time, however, the environmental consequences of dispersion of genetically engineered organisms are far from clear.

The Lindow experiment

On September 17, 1982, Drs Lindow and Panopoulos, scientists at Berkeley, submitted a request for NIH approval of an experiment that would involve deliberate release of genetically altered organisms in the open environment. NIH approval was required because the University of California receives NIH funds for recombinant DNA research.

Neither the government nor the university seriously disputes that an environmental assessment is necessary. The university's contention here is that the environmental consideration given by NIH was equivalent to the necessary environmental assessment that the injunction requires only a document labelled "Environmental Assessment".

We thus fear that the university and the government completely misapprehend the district court's holding and the requirements imposed by NEPA [the National Environmental Policy Act]. The most glaring deficiency in NIH'sreview of the Lindow-Panopoulos experiment is its treatment of the possibility of dispersion of recombinant-DNA-containing organisms. An environmental assessment that fails to address a significant environmental concern can hardly be deemed adequate for a reasoned determination that an EIS [environmental impact statement] is not appropriate.

NIH procedures

The district court also found that plaintiffs were likely to succeed in showing that NIH should have completed an EIS (1) in 1978, when it revised the guidelines to make deli-

berate release possible, and (2) in 1982 or 1983, when it became clear that NIH would be receiving proposals for deliberate release. The district court thus enjoined NIH from approving all other deliberate release experiments. The emphasis on the 1978 revision is misguided for two reasons. First, the policy revision did not irrevocably commit NIH to any decision. Second, significant changes have occurred since 1978, including the maturation of genetic engineering to a point where deliberate release is feasible and imminent. It makes little sense to rest a judgment on *prospective* relief on decisions made several years ago in different circumstances.

The Supreme Court has emphasized that the question of preparing a programmatic EIS [which plaintiffs argued NIH should have prepared in 1982 or 1983] is initially committed to the agency. We are not prepared to agree that plaintiffs are likely to succeed in showing that the absence of a programmatic EIS unreasonably constricts adequate environmental evaluation.

Judge MacKinnon, concurring

I am of the opinion that the foundation should have made its original application to NIH. This is not a case of just failure to exhaust [administrative remedies], but a case of a complete failure of the foundation to present any claim whatsoever to the agency — NIH.

I can understand how the RAC scientists who are knowledgeable in this field of genetic engineering would approve the experiment by a vote of 19-0 with no abstentions. It would seem that an experiment that releases into the environment organisms substantially the same as some already living there, and subject to the same naturally occurring controls, would present no risk. However, the general public and those who have to pass on this action are not knowledgeable in this field and they are easily frightened by new scientific experiments and their possible consequences. It is such lay concerns that must here be satisfied by Environmental Assessments and Impact Statements.

Due notice of the pendency of this matter was given in the *Federal Register* and comment was invited, but none was forthcoming. Had the objections of the foundation been alertly raised before the agency, the district court and this court would undoubtedly have had a better record to consider and might even have been spared the necessity of ruling on the case.

The foundation's conduct has also delayed this vital experiment for a very considerable period of time. The use of delaying tactics by those who fear and oppose scientific progress is nothing new. It would, however, be a national catastrophe if the development of this promising new science of genetic engineering were crippled by the unconscionable delays that have been brought by litigants using the National Environmental Policy Act and other environmental legislation in other areas. □

Star wars

Quarrel over congressional study

Washington

THE Congress's Office of Technology Assessment (OTA) has reacted angrily to accusations that its study of President Reagan's "star wars" Strategic Defense Initiative (SDI) is "designed to kill SDI rather than examine it". The accusations were made last week by Lieutenant General Daniel Graham, a former member of the advisory panel overseeing OTA's study and a prominent champion of space-based missile defences. OTA says there is "no basis whatever" for Graham's attacks and accuses him of breaking faith with OTA's study by distributing confidential documents to senators and to a senior official in an (unnamed) executive agency.

Graham is director of the High Frontier organization, which is widely thought to have been influential in persuading President Reagan to instigate research into missile defences. Graham resigned from the OTA panel on 2 February after being told by OTA's director John Gibbons that he must undertake to keep OTA study documents confidential in order to remain on the panel. Graham admits distributing confidential OTA documents annotated by himself to three senators and the executive official. Graham's explanation for his action is that "this concerns national security and I consider that more important than their damn procedures".

Graham's charges extend both to OTA staff, whose study drafts he describes as "utterly biased", and the advisory panel, which he says is "heavily stacked with highly vocal opponents of SDI". OTA replies that Graham chose to list only those members with whom he personally disagrees; the panel has 21 members in all, chosen to represent all major points of view. Sidney Drell of Stanford University, a critic of SDI who appears on Graham's list, dismisses Graham's charges as "patently false". Another accused panel member, Richard Garwin, praised the work of the OTA staff and the panel chairman. The panel's chairman, Guyford Stever of Universities Research Associates, did not wish to comment on Graham's charges in the interests of maintaining neutrality.

One section of OTA's draft report which particularly incensed Graham lists possible goals of SDI and evaluates them. According to Graham, OTA "stated that a goal is to provide means for the United States to credibly threaten the use of offensive nuclear weapons should it so choose". OTA replies that Graham's quote is taken out of context; the draft did not suggest that this was the actual objective of SDI. Another of Graham's objections is that the report "attempts to make the issue a choice between the Anti-Ballistic Missile (ABM) treaty and SDI".

The issue that brought to a head the row between Graham and OTA was an attempt in the report to separate the issues of anti-satellite weaponry and ballistic missile defence. Graham says this was done to weaken the case for SDI; after unsuccessfully challenging OTA's authority to do so, he took his arguments to senators, and at least one senator has made representations to OTA about the study, says Graham.

OTA is no stranger to star wars controversy; last year, a background paper prepared for the study by Ashton Carter of Harvard University was vilified by Lieutenant General James Abrahamson, director of SDI. Carter's report concluded that the prospect of a near-perfect defence against nuclear missiles was so remote that it should not form the basis of public policy. Graham says he hopes his resignation will serve to discredit the latest OTA report; on the other hand, as one panel member pointed out, it also means there is now one fewer SDI supporter on the panel. **Tim Beardsley**

British cooperation

Washington

FOLLOWING the recent endorsement of SDI research by British Prime Minister Margaret Thatcher during her visit to Washington, negotiations are now under way here to determine how Britain might best cash in on the effort. The SDI office has made it plain that it welcomes allied research cooperation ("we need all the help we can get") and says "the ball's in their court". One major obstacle, however, is that Article IX of the ABM treaty, signed by the United States and the Soviet Union in 1972, expressly forbids the transfer to third parties of anti-ballistic missile technology covered by the treaty. A statement agreed between the signatories makes it clear that the article covers the transfer of "technical descriptions or blueprints specially worked out for the construction of ABM systems or their components". But Britain, and presumably other allied nations, would seek agreements guaranteeing just such tranfers.

The British negotiations in Washington are being led by Dr John Green, deputy head of the embassy's defence staff. One option is that a memorandum of understanding might be exchanged between the two governments; an objective of the negotiations is to establish a coherent framework for British research institutions and companies wanting to offer their expertise to the SDI office. As cost does not seem to be a major object in SDI, there should be plenty of opportunity for qualified British scientists to jump on the bandwagon. But first there will have to be a detailed scrutiny of research capabilities in relation to needs; the results are likely to remain classified. Tim Beardsley

Agricultural research

British council under new threat

THE least favoured of the British research councils, the Agricultural and Food Research Council (AFRC), is faced with yet another period of contraction. At the end of a year in which it has shed 550 jobs, one-sixth of the total, the council is now faced with the prospect of a further reduction of research commissioned by the three agriculture ministries, the Ministry of Agriculture, Fisheries and Food and the ministries for Scotland and Northern Ireland.

This is the gloomy theme of the second of the council's corporate plans, published earlier this week. Sir Ralph Riley, the secretary of the council who retires on 1 April, says in his preface to this document, which will become the basis of the council's claim on public funds in the financial year beginning in April 1986, that a revised plan may be necessary when the government's intentions are known.

The discontent of successive British governments with the council's work goes back at least to an internal inquiry in the late 1960s. At the time of the Rothschild reorganization in 1972-73, AFRC was the most directly affected, being required to depend on commissioned research from the agriculture departments for 75 per cent of its budget. In the past decade, the volume of commissioned work has steadily declined, as has the council's share of the science budget (from which all research councils are supported). Two years ago, the council formally dissented from the annual allocation recommended by the Advisory Board for the Research Councils (ABRC).

In the financial year now ending, AFRC and the Scottish institutes will have received £46.7 million from the science budget and the remainder of the total of £121.6 million by way of commissions. The planned increase of the cash budget for 1984-85 to £125.6 million (not fully compensating for inflation) includes an increase of £3.6 million from the science budget and of £0.5 million in commissioned research.

Government forecasts of public spending published last December show the total budget standing at £126.8 million three years from now (in 1987-88), but the corporate plan says that even this static budget has now been put in doubt by the planned reductions of departmental research and development of £10 million in 1986-87 and of £20 million in 1987-88.

The council is particularly sore at the way in which a Cabinet Office paper published at the end of last year used a comparison of agricultural research costs and output values in the countries of the Organisation for Economic Cooperation and Development (OECD) to demonstrate that Britain spends a greater proportion of the value of agricultural output on research and development than other countries. The assertion was quickly denied (*Nature* 3 January, p.8), and the corporate plan published this week says that other indicators would lead to different comparisons. Yet senior officials of the council fear that the Cabinet Office comparison is still being "hawked around Whitehall" to try to discredit its programme of research.

For the rest, AFRC seems not to have won the moral support it might have expected from the newly-formed Priorities Board on Research and Development in Agriculture and Food, which has apparently been asked to consider what steps could be taken to increase industrial sponsorship for the council's research. The board has apparently taken the line that the central government should pay only for what others cannot be expected to pay for.

The corporate plan says that if the council's budget is further reduced, "further restructuring" may be necessary. During the past few years, this has chiefly meant the closing of research stations and the shedding of staff, but now there is also talk of allowing some parts of the agricultural research enterprise to become free-standing. The possibility that there may be a merger of AFRC and the Scottish research stations is also being explored. □

Planned contraction

THE chief ingredients of the new corporate plan for agricultural research, covering both the work of AFRC and the Scottish stations supported directly by the Department of Agriculture and Fisheries for Scotland are as follows:

University research. The plan is that spending on research grants (increased by 8.5 per cent to £5.4 million this financial year) should continue to take a larger share of total funds. By 1989-90, the plan says, £7 million a year will be spent in this way, with priority given to food research.

Food science. The plan says that AFRC has warmly endorsed the recommendations of the Advisory Council for Applied Research and Development (ACARD) in 1983 that more attention should be paid to food science. Research spending will increase from £13.3 million this year to £19.3 million five years from now, or by 26 per cent in real terms. The plan is to concentrate this work on the three research stations at Bristol, Norwich and Reading, but other council research institutes will also be involved.

Animals research. The intention is that spending on animal diseases should decrease by 16 per cent (to a cash value of £16.4 million a year) over the next five years. Part of the council's recent restructuring has entailed the merging of the Grasslands Research Institute and the National Institute for Research in Dairying at a new site at Hurley, in Berkshire. During the coming year, the Animals Research Institute at Cambridge will be merged with its parent station at nearby Babraham.

Forage crops. The corporate plan outlines a strategy on concentration of research under the umbrella of the Animals and Grasslands Research Institute, with the Welsh Plant Breeding Research Institute and its two Scottish equivalents taking more tightly defined roles within the whole. Plant protection research will decline in the next five years by 16 per cent.

Capital development. The plan says that the recent tendency to spend 12 per cent of the AFRC budget on buildings "cannot be sustained", and that it will in future not be possible to replace some obsolete equipment or even to provide the material support for some new research programmes. But the council plans to earmark £3.5 million from the budget for the year after next to support initiatives not yet proposed.

Manpower. In the year now ending, 100 of the 550 posts lost from the AFRC establishment have been compulsorily vacated. The earlier contraction of AFRC, not yet completed, entails the loss of a further 250 posts during the coming year; the new plan says that it will also be necessary to lose a further 800 posts in the next two years, offset by an increase of 60 posts for food research. The rule that people should retire at age 60 will be strictly enforced, and as many as possible of those not needed will be persuaded to take early retirement. □

Schopper to stay

THE European Organization for Nuclear Research (CERN), near Geneva is to be directed for a further four years by the present incumbent, Professor Herwig Schopper of West Germany. He will thus remain director-general until the end of 1988, which should see the end of the large electron-positron collider (LEP) project.

Italy, which had been campaigning for a strong place in the directorate, has won a place for Professor Carlo Rubbia, Nobellist for his discovery of W and Z particles with his CERN proton-antiproton collider. Rubbia will chair a working group which will study "the long-range scientific and technological future" of CERN.

Meanwhile, after a meeting on the latest proton-antiproton collider results in St Vincent, in northern Italy, last week, physicists were surprised by the continuing discovery of events producing pairs of muons each carrying the same electric charge, but moving in opposite directions. A total of seven such events have now been seen in which the muons are not associated with "jets", the now-familiar signal for quark production and decay. Four of the events have no jets at all, and in five of the seven, strange quarks are produced (double the expected rate). No satisfactory explanation for these "like-sign dimuons" has been offered. Rubbia described these results and others as "a lot of good things cooking". However, "we must be prudent" in their interpretation. Robert Walgate

European research

Commission's new broom

Brussels

KARL-Heinz Narjes, the ex-U-boat officer who is now vice-president of the European Commission and responsible for industry, information technology and science, believes that the Commission's research policy is under test.

"If we fail this test, we will have a major political setback" said Narjes last week, sitting at pentagonal table at which commissioners representing the ten nations of the European Community regularly attempt to plan Europe's future.

A lawyer, and one of the founding fathers of the European Community, Narjes also firmly backs Esprit, the ambitious European programme of research in information technology started by his predecessor, Belgian Viscount Etienne Davignon. At the immense cost (by Commission research standards) of some £80 million a year, exceeding Europe's nuclear fusion research effort for example, Esprit attempts to knock European computer and electronics companies into some kind of coherent research policy and common standards.

Some major companies, such as Olivetti of Italy, have effectively spurned Esprit and believe they can dominate Europe with the help of US allies such as AT&T, while others continue to believe the programme can help (by forcing communication and joint standards). And for Narjes, "certainly Esprit is one of the tests". Moreover, "it is going very well", he claims. "Five hundred researchers are involved this year and there should be 2,000 next, and the first patent has already been applied for."

The setback in December when the European Parliament rejected the Commission's budget for 1985 will not much affect Esprit, the Commission claims, because the bulk of last year's money can be carried forward to 1985. On the other side of this coin, however, it seems the programme has been very slow to get moving.

Nevertheless, the Commission's political masters, the European Council of Ministers, had given a vote of confidence in Commission research programmes on 19 December with agreement in principle to support a substantial expansion of eight major programmes. Biotechnology, for example, will grow from its current budget of £6 million to £30 million a year. The Commission asked council for £2,000 million for 1984-87 and received a promised £1,400 million, still a substantial increase on current spending. "There's relief at being on the move again", said deputy research director-general David Hywell Davies last week, The test, said Narjes, is "whether we can manage the sums allocated".

But Narjes is already looking beyond this "first phase of solidarity" of European research, towards the "horizontal problems". These are the mobility of scientists and "the need to expose them to challenges".

Narjes sees no easy solutions. There are mundane obstacles such as national insurance, pensions, work for a spouse, differing qualification and education systems, security. Language itself is an "underestimated" problem, says Narjes. And it is politically difficult to argue that scientists should be treated as a special case, although the current political interest in using science to solve the economic crisis has given scientists an opportunity that they should seize. "We must make a major, long-term effort" for mobility, says Narjes. Does he have any real hope of success? "We have experience of hard negotiations. We can be 10 per cent tougher than the toughest member state."

Narjes also emphasized:

- The poor access of some non-English-speaking scientists to major US research journals.
- BRITE, a Commission programme to inject new technologies and science into small and medium-scale industries, which will be "a key programme if it succeeds".
- Agriculture, which takes 70 per cent of the Commission's budget "despite serious reforms", where the new biotechnology programme may help to give farmers new economic non-food products.
- The "budget needle", the eye of the needle through which Commission research projects must pass (the total budget still lies around 2-3 per cent of national research programmes). Here "we are seeking other sources" in the Commission, Narjes hinted.
- Big European research machines, such as the politically troubled European Synchrotron Radiation Source, for which the Commission might consider buying a "users' ticket" to distribute through Commission programmes.
- Coordination of Commission research programmes such as BRITE with space station research.
- Intra-Community communications, in science as in other fields, which form "one of the major European problems".

Robert Walgate

European cooperation in the air

BRITE, the European Commission's programme of Basic Research in Industrial Technologies for Europe, may have been oversold by its enthusiastic directors — but it has stolen a march on some other Commission programmes.

According to Dutchman Hendrick Tent, programme head, there has been "overwhelming reaction" to BRITE's invitation to industry and research bodies to participate in projects that will improve production techniques and provide the research base for new product lines in the next 5-10 years.

BRITE has been promised £70 million over four years, to be doubled by industrial contributions, but, like all the new Community research programmes, it still awaits final official approval from the council of ministers of the member states of the Community. This is expected any day now. But BRITE did not delay, having already called for "expressions of interest" to speed up the often bureaucratic process of calling for tenders and choosing and defining final projects. The BRITE team already has a good idea of what projects will be submitted, and has helped companies and researchers to prepare appropriate ideas and consortia, which contain partners from two or more member states and at least one industry. The programme has so far identified a need for projects in reliability and wear, laser technology, joining techniques, testing procedures, computer-aided design, electrochemistry, catalysis and particle technology.

Of the 3,037 expressions of interest received by 31 December, more than half were from industry, and more than a quarter from applied research organizations such as TNO from the Netherlands and the German Fraunhofer Gesellschaft. Fewer than a quarter came from academic institutions, although the proportion was higher in Britain. Britain made the most replies: around 800, compared with 700 from Germany, 500 from France, 400 from Italy, and 100 from Ireland. Some 21 applications came from the Community's newest member, Greece.

- **The European Commission's "stimulation" programme aimed at getting European academic laboratories together rejected 90 per cent of the 700 applicants for its first experimental phase. And it may have to do the same in 1985, the first year of full operations even though its support should increase fivefold (according to the 19 December council of ministers decision).**

So said one of the stimulation programme administrators in Brussels last week, explaining that the demand for European integration at academic level — and the concomitant funds — is enormous.

The stimulation programme should now get at least £33 million for 1985-89 (compared with just £4 million for 1983-84), of which £20 million is to be spent in the first two years, when ministers have encouraged the Commission to reapply for an increase. Sixty per cent of the cash will be spent on the twinning of laboratories, but some cash will also be put aside to run a European version of the "Gordon conference", where a select group of scientists can brainstorm in privacy.

Robert Walgate

NERC's corporate plan

SIR — The leading article (*Nature* 14 February, p. 515) on what you call the prospectus for the Natural Environment Research Council (NERC) contains a good breakdown of our present problems. Your conclusions do not follow. Having made the case for greater cohesion in the field of environmental research and touched on the history of why there is none, the answer is not as you suggest the dismemberment of NERC.

Early drafts of the corporate plan had the idea of submerging the research institutes in the new science divisions. Greater cohesion and flexibility may well come from abolishing the institutes, at the cost of more centralization. The plan published on 14 February was ambiguous. The absence from NERC of mainstream meteorological work is serious, but the corporate plan seeks to include work on climate. The main aim of the plan in its present form is to preserve university grants at a meaningful level. You rightly point out that in five years the universities will hardly notice the benefit. In contrast, in the same period, the funding to institute research could fall by up to 50 per cent and much long-term research will have been written off.

In the British Geological Survey (BGS) many staff feel a new home is necessary. They are committed to the need for continued survey but cannot carry out that task without an adequate level of dedicated funding. BGS finds no stability of finance within NERC and has been the target for an unfair share of cuts already. The proposal that BGS could find an umbrella similar to the British Antarctic Survey is as illogical as it is unlikely. No obvious slot exists for BGS and the present government would never sanction either the making of a new quango or the subsuming of 1,500 extra civil servants into an existing department.

The position of BGS outside central government gives a freedom with which only privatization might compare. The disadvantages of the latter course to the majority of staff and to the scientific needs of the community are obvious.

In the case of the Institute of Terrestrial Ecology (ITE), it is now untrue to say that any greater research links exist with the Nature Conservancy Council (NCC) than with any other government agency with interests in the biological environment. Scientists in ITE see a much wider role in the applied field of pollution studies and all aspects of land use, notably forestry and agriculture. The Ministry of Agriculture, Fisheries and Food and the Agricultural and Food Research Council have been slow to encompass sound ecological principles in answering environmental concerns. The House of Lords Select Committee report on agriculture and the environment (1984) came out in favour of much better liaison in research. Any return of ITE to a functional link with NCC would restrict the research scope and increase the accusation that the institute is simply an advocate of nature conservation. This denies the relevance of ecological work to a whole range of human activities. In any case research should be conducted away from the shackles of departmental policies.

Returning to the leading article of 14 February, your analysis of events leading to the NERC corporate plan was right, but the basic problem is the government's insistence on cuts. As its prescription may not change, it is best to look at what can be salvaged.

I have stated above why for dogmatic reasons the carving up of NERC will not suit the government either. It may be possible to persuade it to accept the proper integration of environmental science in the United Kingdom. In doing so it may get economies and take out some duplicated effort. To allow the fragmentation of NERC would make even more tenuous the links between disciplines and weaken research effort in key areas. This must not happen. Though we are against the planned 30 per cent cut in NERC manpower, we support the need for a properly directed and capitalized research council in the United Kingdom. It is our belief that reduction of institute strength and further fragmentation of research is not the way forward.

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SIR — Given the now unavoidable association between the Natural Environment Research Council (NERC) corporate plan and the notion that a 3,000-strong workforce is to be reduced to 2,000, the choice of St Valentine's Day to announce the contents of such a melancholy document seemed at first sight inapposite until it is remembered that this feast day for romantics also commemorates a notorious massacre.

It may be about to commemorate another one — the sacrifice of the institutes on the altar of the universities. One of the principles underlying the plan and one that appears to have become axiomatic and therefore apparently beyond discussion (see *Nature* 31 January, p.340), is the transferring of institute resources to make up for the shortfall in government financial support for the universities. The plan is obviously modelled on the Mason report into the research councils which made canonical the notion that university research was more flexible and therefore better than the research of the institutes. Nowhere is the term "flexibility" defined or explained. It must be assumed in the absence of an explanation that flexibility in some way refers to an organization's response to the changing times we live in. In other words, it seems flexibility may mean how organizations respond to what has become almost the universal arbitrator — the market forces.

Terrestrial and fresh water ecology seem to have been earmarked for a large contribution towards the "Save the Universities Fund", yet the Institute of Terrestrial Ecology (ITE), for example, was born out of a not dissimilar transfer of funds a decade ago when a substantial percentage of its money was handed over from the Department of Education and Science with the Nature Conservancy Council (NCC) to the Department of the Environment, where NCC was to be a major customer of ITE and any other organization that chose to tender for NCC contracts. ITE has had to compete, and still does compete, with the universities among others for that money. If the universities are not able to obtain enough of that money through competition, are we to understand they will instead be given a proportion of, in this instance, ITE's funds instead? If this is so, how does this principle of flexibility operate other than to appear as a word whose context leaves it without meaning but with a use, namely that of a magic wand with which to shrink institutes.

In place of a more satisfactory explanation of the apparently fundamental concept of flexibility, perhaps there should be much wider consultation, not only on the "details" (*ibid*) but on the principles of the corporate plan.

Otherwise the more cynical employees of NERC institutes may just come to the conclusion that, since the institutes have no direct voice on council and the universities have many, what is being witnessed is not a "rational reconstruction" but something quite different.

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Embryo research

SIR — The question "who can object to the proposition that there can be no such thing as a living being without implantation?" posed in your Opinion column (*Nature* 21 February, p.612) fails to address the objections to embryo research. In my mind the issue is not the point at which an embryo becomes a viable organism, but rather the point at which a unique potential member of our species is created. There can be no objections to the proposition that this takes place at fertilization.

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An appeal to embryologists

The British House of Commons seems bent on passing a bill to prevent some kinds of research. Here is a way in which embryologists and geneticists can help themselves and even help defeat the bill.

MR Enoch Powell's already famous bill to prevent the use of fertilized human embryos for any other purpose than the treatment of infertility seems to have carried the British House of Commons with it. The substantial majority for the second reading of the bill two weeks ago, endorsement of the principle, is an embarrassment for the British government, properly anxious to produce a comprehensive piece of legislation to deal with the problems defined in last year's report from the Warnock committee, but which should also have seen the danger that its plans could be so easily undermined.

What the House of Commons seems not to appreciate is that it has embarked on an unprecedented and dangerous course, the outright banning of certain kinds of research. Its intention, following Mr Powell, is that there should literally never be in Britain even the most sober and well-intentioned investigation of human embryos.

But why should research be sacrosanct, especially when it flatly conflicts with what seems to be an absolute moral principle? This is what the 238 Members of Parliament who voted for the Powell bill were asking two weeks ago. What the scientific community must now recognize is that, while there may be arguments about the force of the moral principle, there is very little chance that this substantial body of opinion in the House of Commons will simply melt away. The practical task is somehow to persuade a large group of sensible people, who must constitutionally be supposed to represent a comparable body of opinion in the general population, that some other course of action than Mr Powell's bill will meet their need.

The starting point must be that research is not sacrosanct. There are already many fields in which investigators do not enjoy untrammelled freedom to do what they like. In most countries, experiments with animals are quite properly regulated with the objective of minimizing the pain that may be suffered in the best-designed experiments. Here the guiding principles are that animals, whose lives are not protected as are those of human beings, must nevertheless not suffer needlessly, which is a goal in its own right but which also protects the investigators concerned from debasement. In genetic manipulation and in work with dangerous pathogens, investigators are free to follow their interests only if they conform with guidelines designed to protect the safety of other people. In most countries, fetal material (as obtained at abortion) can be used, but only with seemly consideration for its source. More to the point, even investigations in which people are the experimental subjects are allowed, but only under the strictest criteria, among which pride of place is taken by the doctrine that the subject must understand what is proposed and must appreciate what risks may be involved. The plain fact is that there are already many fields of research which raise difficult moral problems. In no case has it yet been thought necessary to institute an outright ban. Regulation has been thought sufficient.

The reasons why an otherwise sensible legislature has on this occasion departed from precedent must be understood more clearly. Part of the explanation lies in the title of Mr Powell's bill, the "unborn children (protection) bill". Logician that he is, Mr Powell's argument against regulation rather than an outright ban is easy to predict: embryos are people or potential people who, because of their condition, are unable to give their informed consent. The best hope of winning a better piece of legislation is the likelihood that not many members of the British House of Commons will share this extreme position.

Even a superficial reading of the debate on 15 February in the House of Commons will show that the supporters of the bill span as wide a spectrum of opinion as would be expected. Mr Powell's acknowledged "deep and instinctive" revulsion from investigations of human embryos was widely shared, while many participants in the debate volunteered the opinion that "scientists must be stopped from playing God". The missing ingredient in the debate was the clear statement of what exactly are the benefits of investigations with human embryos. That is not surprising, for as yet there have been no such investigations.

The most common misapprehension seems to have been the almost entire lack of an understanding of what may be meant by research with human embryos. Within the scientific community, it is too easy to suppose that the implications are generally understood. But the chances are that, for many purposes, mere observation (with the help of a microscope) would suffice. Given the general interest in the early differentiation of embryos, it might also be necessary to separate early embryos into individual cells, perhaps so as to understand the circumstances in which totipotency is lost (but those are investigations on which work with other mammalian embryos might suffice). The immunology of the early embryo, and questions of teratogenicity, might involve adjusting the chemical environment of an embryo *in vitro* by the addition of identified substances, may of them naturally occurring. In all kinds of investigations, it would be necessary to be able to dissect an embryo after some fixed period of development, perhaps so as to learn something about its genetic structure. Compared with the more lurid suppositions of what the new opportunities in human embryology constitute — schemes for making literally artificial human beings and the like — this is all dull and almost pedantic stuff. Indeed, except in relation to the technology of *in vitro* fertilization, there seems at present something of a dearth of serious proposals for investigations that would make sense.

Accordingly, those with an interest in these opportunities (and in seeing the debate about the Powell bill take a measured course) are invited to take part in an experiment. The intention is to discover what kinds of investigations with human embryos would, in present circumstances, be carried out. Those willing to participate are asked to send to the editor (in London, in an envelope marked "Embryo Research") a synopsis of a proposed investigation with human embryos, giving a succinct account of the objectives, the reasons for expecting these to be attainable, a brief account of the procedure to be followed (together with an estimate of the numbers of embryos required, controls included) and an explanation why the use of human as distinct from other mamalian embryos is necessary.

Synopses received in the *Nature* office within one month of the date of this issue and judged suitable for the purpose will be sent to a panel of referees composed along the lines recommended by the Warnock committee. Opinions will be solicited from lay people as well as from researchers and physicians. Submissions as such will not be published, but (with the agreement of the authors) the gist of each proposal will appear together with the opinions of all the referees. Those whose submissions are dealt with in this way will be asked to nominate someone (possibly themselves) to receive a copy of *Nature* free for the year ahead. The outcome of this experiment may be to put the Powell bill in perspective. □

Condensed matter and planetary science

First-order phase transitions between amorphous ices

from J.P. Poirier

WHAT seems to be an unprecedented case of a first-order phase transition between amorphous solids is reported on page 76 of this issue. Since it concerns amorphous ices, it has some specific importance for the understanding of icy bodies in the Solar System and for the cryopreservation of biological samples. But it also has general implications for the physics of disorder and for a range of solids that might exhibit transitions between amorphous forms.

Amorphous solids can be obtained by a variety of methods: direct condensation of gases onto a cold plate; quenching of liquids through the glass-transition temperature; and intense irradiation by energetic particles or the impact of high-velocity projectiles. Unlike crystals, amorphous solids are disordered in structure, although not as chaotic as was once thought; they lack crystalline order, but according to the way they are prepared and to the nature of their chemical bonding, different kinds of disorder are found. The physics of ill-condensed matter is an active field of research, in which theoreticians and experimentalists alike are busy describing or predicting the structure of liquids, glasses and all sorts of amorphous solids.

The amorphous ices are of particular interest in two respects. First, they may be present in cometary nuclei, interplanetary dust and ring particles, and in the interior or at the surface of the satellites of the giant planets. Water has at least nine crystalline polymorphic varieties, some of which are metastable far away from their stability field. Second, by the use of cryoprotectants to promote the formation of amorphous rather than crystalline ices, living cells are protected against death caused by the volume expansion that accompanies the normal freezing of liquid water into the common form of ice, ice I. One cannot, therefore, overemphasize the interest of two papers from O. Mishima, L.D. Calvert and E. Whalley of the National Research Council laboratories in Ottawa, Canada. In the first (*Nature* **310**, 393; 1984), they report the preparation of amorphous ice by 'melting' crystalline ice I under pressure at temperatures below the glass transition of water; now, on page 76, they report the phase transition between this and another variety of amorphous ice.

The melting point of ice I decreases as pressure increases, but at low temperature and high pressure other crystalline phases become more stable than liquid water or ice I. The melting curve of ice I can, however, be extrapolated in the stability field of ice VI, showing that, in principle, ice I could 'melt' at 77 K and 10 kbar. By compressing ice under these conditions, Mishima *et al.* obtained an amorphous phase, which persists in metastable state on removal of the pressure. The density of the new phase is consistent with the extrapolated density of water under pressure. At atmospheric pressure and 77 K, the amorphous phase has a density of 1.17; it does not have the same X-ray diffraction pattern as the amorphous phase prepared by quenching water by jet freezing (Mayer, E. & Brüggeller, P. *Nature* **298**, 715; 1982). On heating to 117 K it transforms, with heat evolution, to a less dense amorphous phase resembling the form obtained by condensation of vapour onto a cold plate at temperatures above 80 K. There is clearly a rich variety of amorphous structures and their relationships have to be investigated.

In their paper, Mishima *et al.* report that compression of the low-density (0.94) amorphous ice obtained by vapour condensation produces the same high-density phase as they obtained by 'melting' ice I under pressure. The new and interesting point is that the transition, which occurs sharply at 6 kbar, seems to be the first reported case of a first-order transition between amorphous solids.

The theoretical possibility of phase transitions between amorphous solids or quenched liquids with differing packing structures has been considered by F.H. Stillinger and T.A. Weber (*Science* **225**, 983; 1984). At a meeting last year on Ices in the Solar System (see *Nature News and Views* **308**, 692; 1984) E. Mayer and R. Pletzer reported that vapour condensation onto a cryoplate provides two varieties of amorphous ice: a low-density porous solid when the flow is supersonic and a glass-like, dense non-porous solid when the flow is broken up by a baffle. Their hypothesis about the structures of these phases implies polymorphism for the amorphous ices. It is not yet clear whether the dense amorphous ice produced by Mishima *et al.* is the same as that of Mayer & Pletzer or that reported to be formed by condensation of vapour on a plate at temperatures lower than 10 K (Narten, A.H., Venkatesh, C.G. & Rice S.A. *J. chem. Phys.* **64**, 1106; 1976).

Clearly the evidence for first-order phase transitions between amorphous structures opens exciting new vistas in the physics of disorder, although one may, perhaps, shudder at the thought of the pressure/temperature phase diagram of water, with its superposition of many stable and metastable crystalline phases, being further complicated by the addition of boundaries

Riddle of reflections in the water

WHAT is the shape of the image of the mast of a sailing ship seen reflected in the water? Suppose the surface of the water is wavy, but not to the extent of creating sharp wave-crests (that is, the gradient is nowhere discontinuous). Of course, you will say, the image of the mast will be 'wiggly'. But will there be pieces of mast seen disconnected from the wiggly main image? Presumably yes, since there is no reason why there should be not be an occasional area of water surface off to one side and so inclined that it would throw light from the mast to the observer, and at the same time there might be no area in between that would do likewise.

What shape will such a disconnected piece of the mast image have? In particular, will there be 'ends', where the mast image appears broken? The picture on the cover of this week's *Nature* gives the answer. Disconnected pieces are closed loops. Why?

Here is a simple way to visualize the situation. Suppose instead of the mast we have a vertical line dividing a black area on the left from a white area on the right, each such area stretching out indefinitely far to left and right respectively, as well as upwards. The image area in the water on the right-hand side will then be largely white, but it may contain some black patches. Again, it is clear that they can be disconnected, meaning that they are wholly surrounded by white, since there can be an occasional patch of water surface at such an inclination that it looks into the black side of the object plane. What is the nature of the edge of such a black patch?

We may choose two closely adjacent points in the image, one within the black patch and the other just outside, in the white. Since the water surface has no discontinuities of gradient, the two adjacent rays must come to adjacent points in the object plane. But the only places where black and white areas are adjacent is on the dividing line. Since this consideration applies to every pair of points straddling the edge of our black patch, it implies that its entire edge must image a part of the dividing line into a closed loop, wherever a disconnected patch occurs. The appearance of the sailing ship mast follows from the same consideratons.

There is one additional condition required for all this to be true. The surface of the water must not anywhere be inclined to the horizontal as steeply as the line of sight from the observer, so no area of the surface is shielded from view.

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between metastable amorphous phases. The consequences extend further, as Mishima, Calvert and Whalley point out: other solids, which melt with a decrease of volume (like germanium, silicon and boron nitride), might exhibit several amorphous structures, some of them possibly resulting from 'melting' under pressure at low temperature.

Water vapour in the outer reaches of the Solar System that is condensed as amorphous ice and small bodies, like the particles in the rings of Saturn, the nuclei of comets and small icy satellites, may still contain a large proportion of (presumably) low-density amorphous ice (Smoluchowski, R. *Science* **222**, 161; 1983). Meteoritic impacts on the surface of icy bodies may also form amorphous ices by direct amorphization due to the shock, reversion of shock-produced high-pressure phases, or recondensation of vapourized water. It has been suggested that the heat evolved during the transition from amorphous ice to cubic ice I at 153 K provides the supplement of energy needed to melt and resurface Saturn's satellites Enceladus and Dion (Klinger, J. *Nature* **299**, 41; 1982) and alters the heat and mass balance of comets (Klinger, J. *Science* **209**, 271; 1980). As pointed out by Mishima *et al.*, we now have to take into consideration the possibility that low-density amorphous ice might transform into dense amorphous ice during accretion of the larger icy satellites (like Ganymede, Callisto or Titan) and then retransform exothermally into a variety of amorphous structures as the temperature increases inside the planet. □

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Evolutionary biology

A new phylogeny for Darwin's Galapagos finches

from Paul H. Harvey

EVOLUTIONARY biologists owe it to the memory of Charles Darwin to produce a phylogeny of his famous Galápagos finches. Schluter has produced one such phylogeny in a recent paper[1]; more importantly, he constructs it using a new method which assumes that natural selection alone has caused changes in morphology among the different finch species. Darwin might have appreciated that.

There have been two previous attempts to describe the relationships among the various finch species. The first, by Lack[2], was a qualitative assessment based on comparative morphology. The second, by Yang and Patton[3], used electrophoretic variation of proteins. Differences between the two estimates may result both from the different databases used and the failure to base the analyses on suitable models of evolutionary change.

The justification for Schluter's new analysis comes from recent ecological studies on Darwin's finches by Grant and his colleagues[4,5,6]. Their work leads to the conclusion that morphological variation among the finches is largely the result of differentiation by natural selection. But genetic constraints must also be taken into account if an evolutionary tree is to be derived on the assumption that differences between species are purely a result of natural selection. Genetic variances and covariances among the characters used in the analysis are the two relevant genetic constaints[7]. If genetic variances are high then characters are more susceptible to change by natural selection. If two characters covary positively then more selection is required to increase one and decrease the other than if the genetic covariance were to be negative. Lande[8] produced a quantitative genetic model that takes these factors into account and Schluter has adapted the model in order to estimate the total net force of natural selection that has led to differentiation among Darwin's finches. His analysis is based on phenotypic variances and covariances among eight measures of wing, tarsus and mandible size. The variances and covariances among the characters are similar within ground finches and within tree finches but differ between the two groups, so analyses were performed separately.

Phenotypic measures do not necessarily reflect underlying genetic trends but, fortunately, it appears that they do in the case of Darwin's finches. A recent genetic analysis of variation in the tree finch *Geospiza fortis*, which investigates five of the eight characters used by Schluter, shows that the five phenotypic and genetic variances correlate with a value of 0.98, while the ten covariances have a 0.99 correlation[9]. This allowed Schluter to substitute phenotypic estimates for the genetic variances and covariances.

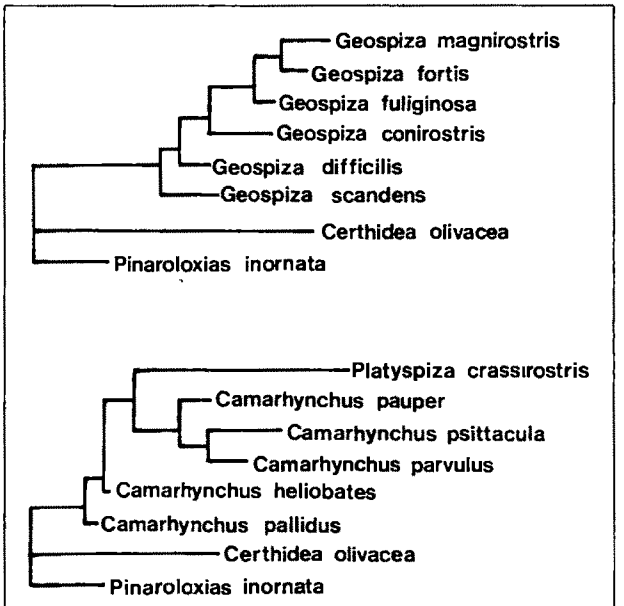
Schluter includes the warbler finch *Certhidea olivacea* in both the ground finch and the tree finch analyses because this species probably branches off prior to the division of the two groups; its omission from the calculations does not change either of the phylogenetic trees. Like Lack before him, Schluter incorporates data from Cocos Island's putative warbler finch *Pinaroloxias inornata*.

The new evolutionary trees, shown in the figure, are similar to Lack's, but there are some differences; the ground finches *Geospiza difficilis* and *G. scandens* appear more closely related than in Lack's analysis, while the tree finches *Camarhynchus pallidus* and *C. heliobates* come close to the ancestral condition according to Schluter, yet are highly modified forms according to Lack. Yang and Patton's electrophoretic analysis resulted in a tree similar to Schulter's, although it is based on only eleven of the fourteen species used by Schluter and Lack.

The new method differs from standard cladistic techniques[10] in measuring branch lengths of the phylogenetic trees, while quantitative variation in the original characters is more effectively incorporated into the analysis. Equally important is the use of distance measures which are based on an explicit genetic model, many of the assumptions of which seem reasonable in the present case. □

1 Schluter, D *Evolution* **38**, 921 (1984)
2. Lack, D *Darwin's Finches* (Cambridge University Press 1947)
3 Yang, S Y & Patton, J L *Auk* **98**, 230 (1981)
4 Boag, P T & Grant, P J *Science* **214**, 82 (1981)
5 Price, T D. & Grant, P J. *Evolution* **38**, 483 (1984)
6 Schluter, D & Grant, P R *Am. Nat.* **123**, 175 (1984)
7 Felsenstein, J *Quart Rev. Biol* **57**, 379 (1982)
8 Lande, R *Evolution* **33**, 402 (1979)
9 Boag, P.T *Evolution* **37**, 877 (1983).
10 Hennig, W *Phylogenetic Systematics* (University of Illinois Press, Urbana, 1966).

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Phylogenetic relationships among the Galápagos tree finches (top) and ground finches (bottom) according to Schluter's analysis. Branch lengths are scaled relative to the length of the *Pinaroloxias inornata* segment.

Stellar structure

Beginnings of asteroseismology

from Douglas Gough

THE science of determining the internal structure of stars from the properties of dynamical oscillations, known as asteroseismology, has only recently become a possibility. Solar five-minute oscillations[1,2] have already yielded important information about the structure and composition of the Sun. Now similar five-minute oscillations have been reported for three distant stars[3-5]. Interpretation of the data from two of these[3,4] provides few surprises, but the third[5] seems to be anomalous and may pose a new and interesting problem to stellar astronomers.

The significant breakthrough came when Isaak *et al.*[2] discovered that they could detect the five-minute oscillations from the Sun in spatially unresolved whole-disk Doppler data. Some spatially resolved data[1], available for several years, had convincingly demonstrated that the five-minute oscillations are a manifestation of resonant acoustic waves trapped beneath the solar photosphere. Although important conclusions about the structure of the Sun's convection zone have been drawn from these observations, such detailed information cannot be obtained from other stars. Whole-disk observations, on the other hand, use light integrated from the entire visible surface of the Sun, which is just what one is forced to do when observing the distant stars.

Oscillations of ordinary stars have the spatial variation of spherical harmonics. For each value of the degree l of the harmonic, there is a sequence of resonant acoustic modes that are labelled with the integer n. These correspond to the fundamental tone ($n = 1$) and the overtones (of order $n-1$) of a musical instrument. On the whole, n is the number of spherical nodal surfaces inside the star, and is a measure of the vertical component of the wave number of the acoustic wave; the horizontal component of the wave number is approximately l/r, where r is the distance from the centre of the star.

A resonant acoustic mode can be considered as an appropriate superposition of many propagating sound waves with the same frequency. Because temperature, and hence sound speed, increase with depth inside a star, downward-propagating waves are refracted back towards the surface. Waves with periods longer than about three minutes, in the case of the Sun, are also reflected by the highly stratified material immediately beneath the photosphere. When l/n is large, the waves are refracted quickly, and are thus confined to a thin outer shell immediately beneath the stellar surface. Its thickness is about $(2n+3)\,R/l$, where R is the radius of the star. When l/n is small, the waves propagate nearly vertically, and penetrate to the core of the star. For the Sun, it has been possible to infer the variation of the sound speed through much of the interior[6] by analysing five-minute oscillations with a wide range of l/n, with a technique bearing some resemblance to that used by particle physicists to infer potentials from scattering experiments.

Whole-disk observations are sensitive to only the lowest values (0-3) of l, and consequently cannot access a very wide range of l/n. Nevertheless, in principle one can still gain useful information about the stellar interior. The density and pressure througout the star can be expressed in terms of linear combinations of oscillation eigenfunctions, with coefficients that depend in a calculable way on the eigenfrequencies, rather like expressing a function as a Fourier series. By measuring oscillation frequencies one might therefore hope to determine how the star is stratified. Indeed, using artificial data it has been demonstrated that if the frequencies of a substantial number of appropriate low-degree solar oscillations are known, the interior pressure and density distribution can be inferred quite accurately, provided the variation of the adiabatic exponent is unimportant.

Unfortunately real data of that kind are not yet available. In the case of the Sun, the acoustic modes are excited to an amplitude great enough to have been detected only in a frequency range between about 2 mHz and 4 mHz (though there is now evidence that another class of modes—the gravity modes, whose restoring force is provided by buoyancy—has also been observed). These modes are quite high overtones, with n between approximately 12 and 33, and by themselves are inadequate for describing the interior stratification—without the lowest sinusoids in a Fourier expansion one cannot in general obtain a good representation of a function. Nevertheless, one can draw some useful conclusions.

The cyclic frequencies ν of low-degree acoustic modes satisfy

$$\nu = (n+\tfrac{1}{2}l+\alpha)\nu_0+\varepsilon(n,l),$$

where α and ν_0 are constants and $\varepsilon/\nu = 0\,(n^{-2})$ when n is large. With the exception of only the lowest-order modes, the frequencies form groups with alternate odd and even l, which are approximately uniformly separated by $\Delta\nu = \frac{1}{2}\nu_0$. A moderately resolved power spectrum of oscillation data should reveal this spacing. It establishes the value of the base frequency ν_0, which is half the reciprocal of the time taken for sound to travel from the star's centre to its surface.

The whole-disk observations first reported by the Birmingham helioseismologists[2] clearly exhibit this property in the oscillations of the Sun, with $\Delta\nu$ of about 67.8 μHz. Now we have the first indications of similar oscillations in other stars. By combining photometric observations of the Ap star HR 1217 over the same time period from South Africa and Chile, Kurtz and Seeman[3] have found six approximately uniformly spaced frequencies near 3 mHz. Fossat *et al.*[5] and Noyes *et al.*[4] also have evidence for uniformly spaced frequencies of α Centauri A and ε Eridani, respectively.

The mean frequency separation measured by Kurtz and Seeman is 34.7 μHz. As Shibahashi[7] has pointed out, the situation is likely to be similar to that of the Sun: modes of both odd and even degree are probably present, so the observed spacing $\Delta\nu$ approximates $\frac{1}{2}\nu_0$. Computations by Christensen-Dalsgaard[8] show that on the zero-age main-sequence, $R\Delta\nu$ is roughly constant. (Main-sequence stars, like the Sun, are at the stage of their evolution where they are powered by hydrogen fusion in their cores). In other words, all zero-age main-sequence stars have the same harmonic mean sound speed. If evolutionary effects are small we can deduce that HR 1217 has about twice the radius of the Sun, a value typical of an Ap star.

The observations of α Centauri A and ε Eridani are particularly interesting because these stars are quite similar to the Sun. Their oscillation amplitudes are much smaller than those of HR 1217, so more sensitive observing techniques were required. Fossat *et al.*[5] observed variations in the intensities at fixed frequencies on the red sides of the Na-D spectrum lines, with the intention of detecting Doppler shifts. Noyes *et al.*[4] observed variations in the profiles of the Ca II-H and -K chromospheric emission lines. Both found evidence for discrete oscillatory modes. After a certain degree of filtering, it was deduced that the envelope of the power spectrum for α Centauri has a broad hump centred at about 3 mHz, like that for the Sun. In neither case was the power spectrum clean enough to identify individual modes unambiguously, so there is room to doubt the interpretation of the data. Nevertheless, the power spectrum of the power spectrum for α Centauri A[5] and the autocorrelation of the power spectrum for ε Eridani[4] both suggest a periodicity in the power, at frequency separations of 81.3 μHz and 86 μHz, respectively.

The approximate constancy of $R\Delta\nu$ found by Christensen-Dalsgaard[8] for the zero-age main sequence is also a property of main-sequence stars with the same evolutionary age, though the value of the constant changes as the stars evolve. The homologous scaling $R^{3/2}\,\Delta\nu$ = constant is approximately satisfied by a star during its central hydrogen-burning phase, even though the changes the star undergoes are not strictly homologous.

As pointed out by Noyes *et al.*[4], their observations of ε Eridani appear to be con-

sistent with these scaling laws. Judged by its strong Ca II emission, the star seems to be younger than the Sun, and Lacy[9] has established that $R/R_\odot = 0.81 \pm 0.08$, where $R_\odot$ is the present radius of the Sun. Since $R\Delta\nu/R_\odot$ is calculated to be 72.5 μHz for the zero-age main sequence, and $\Delta\nu$ is about 68 μHz for the Sun now, one would expect $\Delta\nu$ for ε Eridani to be 84-90 μHz. This is indeed the case.

The observations of α Centauri A do not conform to this pattern. The star is a member of a binary system and is known to have a mass of 1.1 $M_\odot$. According to Christensen-Dalsgaard and Frandsen[10], $\Delta\nu$ should have been 71 μHz when such a star was on the zero-age main sequence; its radius should have been 1.03 $R_\odot$. Since $\Delta\nu$ is now 81.3 μHz, one would deduce from homologous scaling that R is approximately 0.94 $R_\odot$. Having a higher mass and apparently a lower radius than the Sun, at first sight α Centauri might therefore also be thought to be younger because, according to the theory of stellar evolution, the radii of late-type main-sequence stars increase with both mass and age. But the observed $\Delta\nu$ appears to imply that α Centauri A has shrunk. This contradicts observation as well as theory, since Blackwell and Shallis[11] find from the infrared magnitude and a knowledge of the distance of α Centauri that $R/R_\odot = 1.23 \pm 0.04$. Moreover, Flannery and Ayres[12] conclude, from matching the theory of the evolution of the companions of the binary system with all the available observations, that α Centauri is probably older than the Sun. Either the oscillation data have been misinterpreted or the structure of α Centauri A is quite different from expectations.

More highly resolved measurements of the oscillations will have to be made in order to decide between these possibilities. It would help to have several days of almost continuous data, which can be obtained by observing from two or more sites widely separated in longitude over the same period, as has already been done for HR 1217 and the Sun[3,13]. Alternatively the stars might be observed from Antarctica or from space. From such data, it may also be possible to measure the small quantity ε in the dispersion relation, which separates the frequencies of modes with like $n + \frac{1}{2}l$ but different l, and which measures conditions close to the centre of the star[14]. This would give us direct information about the stratification of the energy-generating core of a distant star, an exciting prospect indeed. □

1. Deubner, F L. *Astron Astrophys.* **44**, 371 (1975)
2. Claverie, A *et al* in *Nonradial and Nonlinear Stellar Pulsation* (eds Hill, H.A. & Dziembowski, W.A , 181 (Springer, Heidelberg, 1980), *Nature* **282**, 591 (1979)
3. Kurtz, D.W. & Seeman, J *Mon Not R astr Soc* **205**, 11 (1983)
4. Noyes, R W *et al Astrophys. J.* **285**, L23 (1984)
5. Fossat, E *et al Comptes Rendus Acad. Sci. Paris, Ser II* **299**, 17 (1984)
6. Christensen-Dalsgaard, J *et al Nature* (submitted).
7. Shibahashi, H *Mem Soc astr Italiana* **55**, 181 (1984)
8. Christensen-Dalsgaard, J in *Space Research Prospects in Stellar Activity and Variability* (eds Mangeney, A. & Praderie F) 11 (Paris Obs Press, 1984)
9. Lacy, C H. *Astrophys J Suppl* **34**, 479 (1977)
10. Christensen-Dalsgaard, J & Frandsen, S *Solar Phys* **82**, 469 (1983)
11. Blackwell, D E & Shallis, M J *Mon Not R astr Soc* **180**, 177 (1977).
12. Flannery, B.P. & Ayres, T.R *Astrophys J* **221**, 175 (1978)
13. Claverie, A *et al Mem. Soc astr. Italiana*, **55**, 63 (1984).
14. Gough, D O *Phys Bull* **34**, 502 (1983)

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Scrapie

Perspectives on prions

from Colin Masters

AFTER decades of research into a group of 'slow virus' diseases, Stanley Prusiner and his collaborators have published a continuing series of papers identifying a new type of infectious particle—the prion (for proteinaceous infectious particles). The remarkable resistance of the prion to chemical and physical procedures that attack nucleic acids, and its small size (reportedly 100 times smaller than the smallest virus) have directed attention towards the possibility that the prion consists of protein without associated nucleic acid and that its replication proceeds via protein-directed protein synthesis—a situation which would be without precedent in biological science and contrary to the dogma that genetic information invariably flows from nucleic acids to proteins. Consequently, it is not surprising that the research (see refs 1 and 2 for reviews) has given rise to a great deal of speculation on the nature of prions, on the alternative possibilities of their replication, and on their significance in relation to several chronic degenerative diseases of humans. The potential ramifications make it worth assessing the status of prion research.

The research may be traced back to the 1950s when the first intensive studies began in the highlands of New Guinea into the strange degenerative condition known as kuru[3]. The dramatic nature of these findings (kuru was found to be transmitted by the cannibalistic habit of eating uncooked brain) stimulated interest in scrapie, a related disease of sheep and goats which can be produced experimentally in small laboratory animals. Scrapie researchers have coped with many formidable technical difficulties but after experimental models of scrapie were produced in mice and hamsters in the 1960s, several groups began to purify the causative agent[2,4,5], which was presumed to be a virus. It became apparent that the agent was firmly associated with cell membranes and could only be released by quite drastic treatment. Proteases, detergents and denaturants were commonly used in purification and it was the remarkable stability of the infectious agent to these substances, together with its resistance to nucleases and ultraviolet irradiation that led to a view that the scrapie agent may not be a virus at all.

Much of the data pointed to the importance of the protein content of the particle for scrapie infectivity. This, together with the continued failure to demonstrate the existence of a scrapie nucleic acid, led to the suggestion that the protein may either code directly for its own replicative synthesis or induce genes of the infected animal to do so for it[1]. Subsequently, similarities between prion aggregates in tissues and the amyloid plaques that are features of several human diseases were pointed out by Prusiner, who suggested the involvement of prions in a wide range of conditions including senile dementia, multiple sclerosis, rheumatoid arthritis, Parkinson's disease, diabetes, lupus erythematosus and cancer[2]. Clearly, if these postulates are substantiated, they will have momentous implications for biomedical science.

What remains to be done then before this critical and highly contentious situation is finally clarified? At present, quite severe criticisms of major elements of the prion hypothesis are still appearing in the scientific literature[6,7]. Several authors have pointed to the fact that much of the evidence is negative in nature—never a fully satisfying situation for the scientist. Much of the data on the physiochemical characteristics of the infectious agents in tissues is difficult to interpret because of the crudity of the extracts. It has been suggested, for example, that the organism's extraordinary resistance to inactivation, which has led to much of the present speculation on this new life form, may not be a feature of the overall population of particles but rather is limited to small subpopulations. It has, moreover, been claimed that when conventional viruses are suspended in tissue homogenates, they too can provide examples of resistant populations and insensitivity to virucidal agents. The deduction that the scrapie agent has a sub-viral size has also been questioned, and it has been pointed out that there is good *a priori* evidence that the scrapie agent has a nucleic acid genome, even if direct biochemical evidence is lacking. Finally, it has been argued that if all the scrapie agent has to do is be replicated by host enzymes and interact with the host in strain-specific ways to produce disease, its nucleic acid could be unusually small and difficult to detect.

Clearly, it would be possible to resolve most of the outstanding questions if the infectious agent were available for examination in purified form. It is, therefore, encouraging to note that rapid progress is being made in that direction. It

has been established that the bulk of the protein in the scrapie agent consists of a single molecular species (called PrP, for prion protein)[8]. PrP is a glycoprotein, of apparent relative molecular mass of 30,000, and has been highly purified and partially sequenced. When the complete sequence has been determined, it will be possible to synthesize the same sequence and test whether it has the same biological action as the natural agent. Only then will it be determined with certainty whether prions are both infectious and devoid of nucleic acids. When the synthetic molecule is available, antibodies to it could also be produced for titration of scrapie, and synthetic sequences of the corresponding gene could be constructed and used to investigate both the function of the PrP gene in the cell and the presence of the PrP gene in the host genome. It will also be important to determine the amino-acid sequences for myeloid proteins and compare them with PrP to establish the exact connection between amyloid plaques and the infectious agent.

The imminent resolution of many of these questions heralds an exciting era of biomedical advance. An intriguing area of scientific curiosity which has been impeded by forbidding technical problems has been transformed into a rapidly progressing and productive area of contemporary research. Already, the the immunological evidence available appears to provide support for the view that the amyloid plaques in the brains of scrapie-infected animals and humans with Creutzfeldt-Jakob disease are composed of arrays of prion proteins[9]. Soon we should know for certain whether prions do indeed represent a revolution in molecular biology and medicine, or if they are another manifestation of the complexity of interpreting molecular behaviour in the cellular microenvironment. □

1 Prusiner, S B *Science* **216**, 136 (1982)
2 Prusiner, S B. *New England J. Med* **310**, 661 (1984).
3. Gadjusek D L *Science* **197**, 943 (1977)
4. Alper, T., Haig, D.A & Clarke, M C. *Biochem. biophys Res Commun* **22**, 278 (1966)
5 Alper, T., Cramp, W A , Haig, D A. & Clarke, M C *Nature* **241**, 764 (1967).
6. Rohwer, R G *Nature* **308**, 658 (1984)
7 Kimberlin, R.H *Trends biochem Sci* **7**, 392 (1982)
8. Prusiner, S B., Groth, D F., Bolton, D.C , Kent, S B & Hood, L.E *Cell* **38**, 127 (1984)
9 Brockman, J M *New Eng J. Med* **312**, 73 (1985).

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Immunology

Control of T-cell development

from Roland Scollay

OUR understanding of the control of the proliferation and early differentiation of T lymphocytes in the thymus gland is hazy, to say the least. Yet the thymus supports a large population of dividing cells[1] and about 30 per cent of its lymphocytes are replaced every day[2]. The hope that either one of the thymic hormones or interleukin-2 (IL-2) — a potent growth factor for mature stimulated T lymphocytes — would turn out to be the key factor for proliferation of the blast cells from which T lymphocytes are derived, has not been realized[3,4]. As a result the notion has arisen that growth factors are less important for proliferation than the interaction between blast cells and other non-lymphoid cells of the thymus. But two papers in this issue[5,6], showing IL-2 receptors to be on a subset of cells in the thymus, come as a faint ray of hope that IL-2 is the key.

About 80 per cent of the blast cells (15 per cent of all cells) in the murine thymus are the parents of the major population of small thymocytes in the cortex of the thymus. On their cell surfaces, they carry two antibody-defined markers associated with functional T lymphocytes[7]. The markers are Ly2 and L3T4, so the cells are classed 2^+4^+. Most of the other blast cells (about 3-5 per cent of all thymocytes) are 2^-4^-— that is they express neither Ly2 nor L3T4 and are generally felt to be precursors of, presumably, both cortical and medullary cells[7]. These 2^-4^-blasts are the predominant population in the embryonic murine thymus from day 12-16 or 17 of gestation, an observation which lends credence to their role as precursors[8].

It is on these 2^-4^-blasts that receptors for IL-2 have now been demonstrated. Ceredig *et al.*[5] use an antibody to the IL-2 receptors to show that about half the 2^-4^- blasts in both embryonic and adult thymus express the receptor, although those from the adult thymus (embryonic not tested), are slightly fewer in number and lower in affinity than those found on mature lymphocyte (Con A) blasts. They also demonstrate that the cells with IL-2 receptor are scattered throughout both the cortex and medulla of the thymus. Using different monoclonal antibodies, Raulet[6] finds a very similar distribution of the receptor on adult and embryonic blasts but, unlike mature lymphoid blasts which respond vigorously to IL-2, neither population of thymic blasts responds to IL-2 unless an additional stimulus (Con A) is added.

These data are relevant to a number of issues. First, it has generally been felt that 2^-4^-blasts are mainly confined to the outer cortex of the thymus, even though blasts in general, although more concentrated in the outer cortex, are widely distributed. The data from Ceredig *et al.*[5] show that the IL-2 receptor-positive 2^-4^- blasts are also widely distributed. Whether 2^-4^-cells occur in the medulla is still unclear, since it has not been established whether all the IL-2 receptor-positive cells are 2^-4^-, as both papers point out.

The second but related point concerns the heterogeneity of 2^-4^-cells. Other work shows that they are not a homogeneous population. Are receptor-positive and receptor-negative cells precursors for two pathways or lineages, or are they sequential stages of one (or more) pathway? The new data open up several interesting lines of experiment to answer such questions.

The third issue concerns the role of the receptor in cell differentiation. The receptors on thymic 2^-4^- blasts have relatively low affinity for IL-2, which perhaps accounts for the fact that the cells are not well stimulated by IL-2 alone. Unfortunately, there is no affinity data for the embryonic cells, which respond even less well to IL-2 alone, so it remains unclear whether this reflects a real difference in receptors or a response by the unkilled mature cells in the adult 2^-4^-preparation.

Nonetheless, the presence of the receptor in the embryo is important in interpreting the adult data. In general, immunologists associate IL-2 receptors with antigen- (or mitogen-) activated T lymphocytes and with mature cells that produce IL-2. The fact that before day 16 the embryo seems not to express antigen receptors[9] and that neither IL-2 producing cells nor cells of the usual (mature) IL-2 producing phenotype are detectable until several days later[8], suggests that the receptor on 2^-4^- cells may be operating quite differently from those on mature T cells. Indeed, the whole process within the thymus must be quite different, since the major, rapidly-dividing 2^+4^+ blast population, which presumably derives from the 2^-4^- blasts, is completely lacking the receptor[5,6] unlike any other population of activated lymphocytes. Perhaps we should not be unduly influenced by our knowledge of the activation process in mature cells in considering the significance of the IL-2 receptor on thymic cells and its presence on cells should not necessarily be taken as evidence that they are stimulated by antigen.

Some evidence of IL-2 production in the embryo would go far to establishing the relevance of the receptor. Without this, I find it too early to speculate on the receptor's role. But clearly, these experiments and the rapid increase in knowledge of the T-cell receptor brings us closer to an understanding of the secret workings of the thymus. One day a cocktail of factors added to a single T-cell precursor may allow us to observe the complete process of T-cell development in a test tube; but it may yet turn out that cell-to-cell interactions are essential. □

1. Scollay, R & Shortman, K. *Thymus* **5**, 245 (1983).
2. Bryant *Eur. J Immunol.* **2**, 38 (1972)
3 Andrews *et al. Cell Immunol.* (in the press)
4. Draber, P & Kisielow, P *Eur. J. Immunol.* **11**, 1 (1981).
5 Ceredig, R. *et al Nature* **314**, 98 (1985)
6. Raulet, D.H *Nature* **314**, 103 (1985).
7 Scollay, R *et al. Immunol Rev* **82**, 79 (1984)
8 Ceredig, R *et al. J. Exp Med* **158**, 1654 (1983).
9 Snodgrass, H.R. *et al Nature* **313**, 592 (1985)

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Periodic extinctions

Cratering theories bombarded

from Paul Weissman

ONE of the first lessons for any young scientist is how to limit research to a finite task. "All of us", wrote geophysicist E. C. Bullard in 1960, "dig our own small specialized holes and sit in them". For those scientists who venture out of their holes, few problems today are so quick to bring on feelings of agrophobia as the study of biological extinctions of species on the Earth. Once relatively confined to the planet, the various aspects of the extinction problem have reached out first into the Solar System, and now to the Galaxy surrounding it. To explore these new horizons, astronomers and other concerned scientists met in January* to examine, and heatedly debate, the possible interactions between the Earth, the Solar System and the Galaxy.

The problem of biological extinctions was once the sole province of the palaeontologists and biologists who studied the fossil record of the rise and fall of species. Then, in 1980, physicist Luis Alvarez and his geologist son, Walter, along with colleagues, suggested that the Cretaceous-Tertiary extinction 65 Myr ago was associated with the impact of a 10 km diameter asteroid. They found a strong concentration of iridium in the boundary clay coincident with the extinction. Iridium is very rare in the Earth's crust, most of it having gone into the core when the planet differentiated.

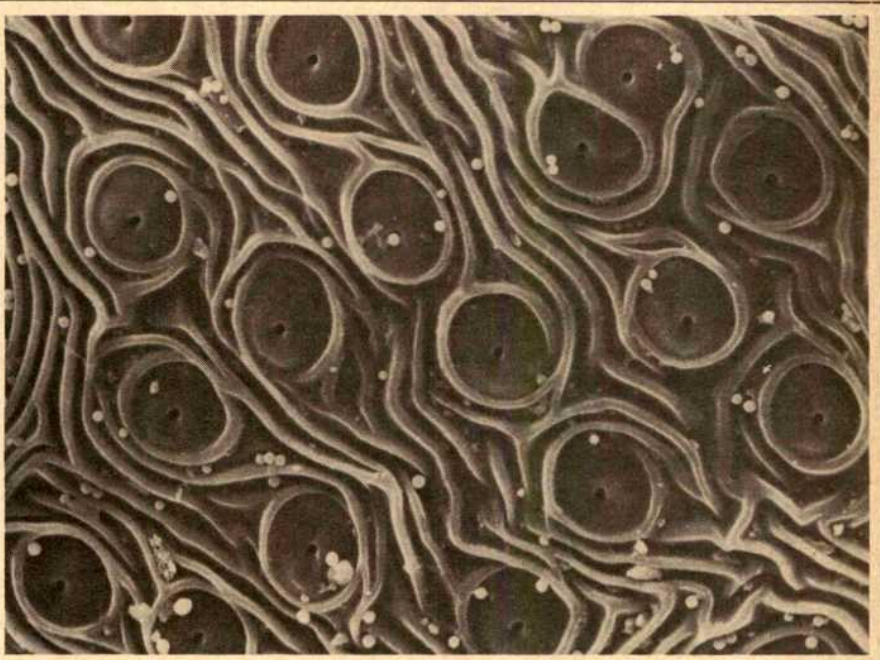
A second expansion of interest in extinctions has followed last year's report by David Raup and John Sepkoski that the extinctions of marine animals over the past 230 Myr seems to have a 26 Myr periodicity. This sparked a number of hypotheses for generating periodic showers of comets from the Sun's Oort cloud — 10^{12}–10^{14} comets surrounding the Solar System and extending out to interstellar distances. Other hypotheses involved a possible association of extinctions with the Sun's harmonic motion above and below the galactic plane, which has a half-period of 33 Myr. Analysis also indicated a possible 28 Myr period in the record of large, well-dated impact craters on the Earth.

One of the first items called into question at Tucson was the reality of the 26-28 Myr periods in the fossil and crater records. Michael Rampino and Richard Stothers (Goddard Institute for Space Studies) reported a variety of periods, ranging from 12-36 Myr, from a reanalysis of the data, and similar periods in episodes of low sea levels on the Earth, geomagnetic reversals, and major tectonic episodes. They suggested that the periods are all harmonics of the Sun's 33 Myr interval between galactic-plane crossings and that a weakly-detected period of about 250 Myr is associated with the mean time between the Sun passing through galactic spiral arms.

Critics of these analyses pointed out that Rampino and Stothers had not identified any mechanisms for relating the various geophysical events to the Sun's z-motion. It has been suggested that the probability of the Sun encountering a galactic giant molecular cloud increases sharply as the Sun passes through the galactic plane, every 33 Myr. But Patrick Thaddeus and Gary Chanan (Goddard Institute for Space Studies) showed that such clouds were well dispersed above and below the galactic plane, and that encounters would occur at essentially random times (see page 73 of this issue). In addition, Piet Hut (Institute for Advanced Study, Princeton) and Scott Tremaine (MIT) presented a detailed analysis showing that galactic giant molecular clouds are not likely to be major perturbers of the Oort cloud, contrary to the idea of Biermann and Lust in 1978 and strongly argued for ever since by Victor Clube (University of Oxford) and William Napier (Edinburgh Oberservatory).

Gene Shoemaker (US Geological Survey) reported a reanalysis of the cratering and extinction records, with a best fit period of 31-32 Myr. But he pointed out that the dating for many of the extinction events is extremely poor, expecially beyond about 140 Myr ago. Shoemaker also noted that because most workers is this field now accept the idea that impacts and extinctions are related, the fossil and crater records are not independent, and might well agree in both period and phase of any pseudo-periodicity. As compelling as the evidence may seem, Shoemaker characterized it as 'shot noise' — simply a sequence of very few random events arranged with apparent periodic spacing. Support for this view came from Tremaine, who generated several hypothetical histories of random cratering and then challenged the audience to distinguish the real history from the 'best' random sample. About one-fifth of the assembled scientists chose the random sample. Tremaine then showed that statistical analysis in the random data gave a better fit to the 26-Myr period than the actual cratering history.

All this did not deter the proponents of the several mechanisms for creating a 26-Myr periodicity in showers of comets from the Oort cloud. The idea of an unseen solar companion star with a 26-Myr period has been suggested independently by Daniel Whitmire (University of Southwestern Louisiana) and Albert Jackson (Computer Sciences Corporation), by Marc Davis and Richard Muller (University of California, Berkeley), and by Piet Hut. The unseen star, presumably of low mass, would have to be in an orbit with a semi-major axis of about 87,000 astronomical units (1.4 light years) to have a 26-Myr period, but with a high eccentricity, of 0.7 or more, so that the perihelion distance is close to the inner Oort cloud where the comet density is highest. Dynamical studies by Hut, Mark



Pustuliform organs on the abdomen of the spider *Holcolaetis vidua* Lessert, magnified × 880. It may be that pheromones are secreted through the pore in each organ, and it is not inconceivable that the spherical bodies associated with, or even lodged in, the pores are 'packets' of pheromones. Mytiliform organs on the abdomens of Spartaeinae spiders may serve the same function. The two types of organ are described and compared by F.R. Wanless in the course of a revision of the spider genus *Cyrba* in the Bulletin of the British Museum (Natural History), Zoology Series 47, 445 (1984).

*"The Galaxy and the Solar System', Tucson, Arizona, 10-12 January 1985.

Bailey (University of Manchester), and Jack Hills (Los Alamos) showed that such an orbit was unstable and would be likely to escape from the Solar System in less than 1.0 Gyr. Since capture of a companion star is extremely unlikely, the orbit must have been more tightly bound in the past.

The Berkeley group believes that the last periodic extinction was about 12–14 Myr ago, so the proposed companion star, which they call Nemesis, is now near aphelion. Muller described an optical survey of 5,000 low-mass red dwarf stars, which is expected to examine all candidate stars visible from the Northern Hemisphere by the end of this year, and will be extended to the Southern Hemisphere in 1986. Nemesis, if it exists, might also be detected by the all-sky survey of the Extreme Ultraviolet Explorer Satellite, scheduled to be launched in late 1988.

Whitmire and John Matese (University of Southwestern Louisiana) suggested an alternative mechanism for creating periodic cometary showers that involves a tenth planet, some 100–150 AU from the Sun. They suggest that the orbit of the planet is eccentric and highly inclined, and that it has swept a 'clear zone' out of the inner ring of the Oort cometary cloud. As the orbit precesses, however, it will occasionally come close to the edges of the clear zone and again perturb the comets there. The comets are first thrown into Jupiter-crossing orbits, and then some fraction of them reach the Earth's orbit. Comets which do not hit the Earth are eliminated by Jupiter's perturbations in a period of about 1 Myr. Whitmire and Matese believe that the tenth planet has a mass one and a half times that of the Earth and an orbit inclination of at least 45 degrees.

I raised a number of objections to the hypotheses involving cometary showers. Current estimates of the cratering rate on the Earth and Moon from known craters on dated surfaces agree well with expectations from the observed flux of Earth-crossing comets and asteroids, whereas adding intense comet showers every 26 Myr would raise the cratering rate by a factor of 5–18, inconsistent with the observed craters. The only way out of this dilemma is to assume that the Solar System is currently in the midst of a cometary shower, and that the 'steady state' observed cometary and asteroid flux (many dynamicists hold that the Earth-crossing asteroids are extinct comets) is anomalously high. This might work with the 31–33 Myr period, which puts the last periodic extinction only 4 - 5 Myr ago, but not with the 26 Myr period, which sets the last extinction some 10 Myr farther back in time. Moreover, analyses of impact melts from terrestrial craters suggest that many were made by highly differentiated meteorites, like irons and achondrites (similar to basalts). This is inconsistent with a cometary source, since comets are believed to be the most pristine undifferentiated objects in the Solar System, a conclusion supported by analyses of interplanetary dust collected by high-flying aircraft.

The advocates of cometary showers countered that the identifications of the objects that formed the crater are still very tentative, and that the flux of comets in a shower might be up to an order of magnitude less than their original estimates, thus lowering the predicted cratering to acceptable levels. The question of whether the lower fluxes would also decrease the probability of major biological extinctions was not answered.

In the end, no hypothesis for or against periodicity emerged as clear winner, and few converts were made, but a very convincing picture emerged of the ways in which the Galaxy could affect the environment within the Solar System and on Earth. Although the conference participants did not come to any clear concensus on the extinction problem, they returned home with broadened horizons and, perhaps, with the temptation to continue to explore beyond their specialized holes. □

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Biogeology

Sulphur-eating tube worms take to the Oregon breaches

from Roger N. Anderson

SCIENTISTS attending meetings of the American Geophysical Union (AGU) have grown accustomed to new reports of undersea oases of brilliant red tube worms and giant red-meated clams clustered around black smokers, which billow forth 400°C hydrothermal fluids at the axes of mid-ocean ridges. New communities from the eastern Pacific have been reported with remarkable regularity since their initial discovery in 1977. More were reported at the AGU winter meetings in San Francisco (4–9 December 1984), but there was a big surprise in store. A series of four consecutive papers[1-4] documented the presence of sulphur-eating tube worms and giant clams in a completely new environment: on the accretionary pile of sediments found above a subduction zone, where there are no hot springs.

The biological communities were found living along 'breaches' or open rifts that were discovered during dives of the submersible *Alvin* along a series of anticlinal folds of newly accreted sediments on the continental margin off Oregon, in the north-west United States. No hot-water springs were detected by the sensitive thermometers on *Alvin*, but methane-enriched waters were captured. These, along with methane-derived calcium carbonate cementation found within the surface sediments, verify that sulphur-rich waters are being expelled along the breaches. Methane and sulphur are both added to hydrothermal waters during chemical interaction with basalts of the oceanic crust.

Though not associated with hot springs, the methane-rich waters of the breaches probably have a source similar to that at ridge crests. The subduction of the Juan de Fuca plate under the coast of Oregon is the likely source of the sulphur and methane. Waters circulating in the crust eventually end up as pore fluids in sediments being accreted to the west coast of Oregon. Dewatering of these sediments is apparently a more dominant physical process than previously thought, since sufficient flow must be occuring to supply enough nutrients to support the same kind of biological community as that living near ridge-axis black smokers. Contrary to popular belief, these new observations show that hot temperatures are not required to support the organisms.

The typical sedimentary diagenesis that is observed in the area points to widespread venting all along this margin. The microcrystalline dolomite, magnesian calcite, and aragonite that surround the vents result from methane reactions. Such mineralogy is common throughout older accretionary complexes of the world. Dewatering of 20 per cent by volume of the accretionary sediments is required to support the volume of diagenetic sediments observed off Oregon. The dewatering becomes more pronounced as one proceeds away from the trench axis toward the shoreline. Tube-worm communities were sampled on vents in sediments accreted up to 1.8 million years ago.

These discoveries, and another report[5] of similar sulphur-eating organisms at the foot of the Florida escarpment, where sulphide-rich hypersaline waters are seeping out at ambient temperatures onto the sea floor 3 km below the surface, show that our tube-worm friends are more plentiful than ever anticipated. The joy of all this for geologists is that we can learn much about subsurface hydrological processes while observing some of nature's true wonders. □

1. Carson, B., Ritger, S.D. & Suess, E. *EOS* **65**, 1089 (1984).
2. Kulm, L.D., Thornburg, T.M. & Carson, B. *EOS* **65**, 1090 (1984).
3. Moore, J.C., Kulm, L.D., Lewis, B.T.R. & Cochran, G. *EOS* **65**, 1089 (1984).
4. Suess, E. & Mossoth, G.D. *EOS* **65**, 1089 (1984).
5. Paull, C.K. *et al. Science* **226**, 965 (1984).

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Genetics

New aid to human gene mapping

from C. Thomas Caskey

ALTHOUGH the chromosomal localizations of the genes for Duchenne muscular dystrophy[1] and Huntington's disease[2] stand as powerful examples of the use of restriction fragment length polymorphisms (RFLP) to map human genes, considerable technical difficulties still limit the application of the technique. Help is at hand with the report on page 67 of this issue[3] of a new family of DNA sequences, the characteristics of which are highly suited to RFLP linkage analysis.

Typical RFLP analysis has employed cloned functional gene sequences[4], pseudogene sequences[5] or 'anonymous' genomic probes[2], to detect what are presumed to be single base-change polymorphisms that generate or remove recognition sites for restriction endonucleases, the enzymes used to fragment DNA. Alternatively, these probes have been used to detect polymorphisms that are presumed to be due to DNA rearrangements[6]. With the exception of the last, these probes suffer from low heterozygosity because only two alleles are present in the population, one of which often occurs at a low frequency. As a result, no information can be obtained from some critical individuals in a pedigree or even entire pedigrees. Another problem is that genomic recombinants often contain highly repeated sequences that must be removed prior to analysis. Finally, closely linked polymorphic probes may not be available for a chromosomal region of particular interest.

Several of these difficulties should be obviated by the use of the DNA sequence family that is both tandemly repeated and dispersed in the human genome, and is described by Alec Jeffreys and his colleagues in this issue[3]. This family offers the opportunity to recognize multiple unlinked chromosomal loci and, with use of appropriate restriction endonucleases and specific probes, to identify variations in the number of copies of the tandem 'minisatellite' at each locus. The variation in copy number is seen as an RFLP by standard Southern blotting techniques and some loci are shown to be highly polymorphic for copy number of members of this repeat family (multiple alleles).

The minisatellite was first detected during characterization of the human myoglobin gene by Jeffreys and colleagues[7] as an intron sequence that contains four tandem repeats of a 33 base-pair subunit and bears similarities to highly polymorphic sequences that are found near several other genes[8,9]. A probe constructed as a concatamer of this repeat has now been used to identify numerous genomic lambda clones which contain sequences homologous to the repeat. Eight of the clones were selected and further characterized. They differ in their base sequence, number of repeat units and, apparently, in their localization within the human genome (that is, they are not closely linked). All the repeat units, however, share a similar 'core' of 10-15 base pairs. Three of the eight repeats studied are highly polymorphic in copy number when hybridized under high stringency to DNA from various individuals. Pedigree analysis by Southern blotting shows that bands representing different copy numbers of repeats are inherited in a Mendelian fashion. Furthermore, the heterozygosity of these highly polymorphic repeats appears to be close to 100%. Thus, it is highly likely that these probes can yield information for analysing the pedigrees of families affected by a closely linked disease gene.

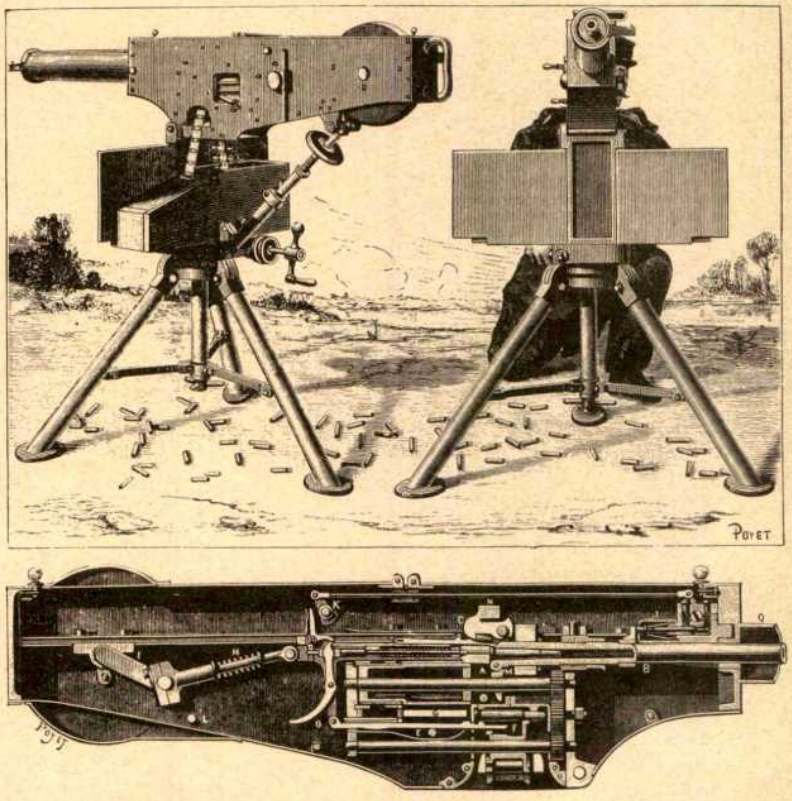
In order for these probes to be useful the chromosomal localization of the minisatellites will need to be determined. But it is clear that they provide an interesting and potentially significant improvement in the use of RFLP's for prenatal diagnosis, linkage studies, and gene mapping. Other polymorphic satellite DNA probes have been reported[10,11], but they do not seem to have the same widespread potential in human gene mapping. Minisatellite sequence families offer the advantages of multiple chromosomal localization and greater heterozygosity at a given locus. Jeffreys *et al.* have described one, but their report holds out hope that other minisatellite families, which would make useful polymorphic probes available throughout the genome, await discovery.

Because the 'core' sequence of the repeated units resembles the generalized recombination signal of *Escherich coli*, a further use of these minisatellite sequences may be in the study of unequal crossing-over, the presumed mechanism for generating heterogeneity of copy number within a tandem repeat. □

1. Murray J.M. *et al. Nature* **300**, 69 (1982).
2. Gusella J.F. *et al. Nature* **306**, 234 (1983).
3. Jeffreys, A.J., Wilson. V. & Thein, S.L. *Nature* **314**, 67 (1985).
4. Nussbaum, R.L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4035 (1983).
5. Patel *et al. Somatic Cell and Molecular Genetics* **10**, 483 (1983).
6. Wyman, A. & White, R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6754 (1980).
7. Weller, P., Jeffreys, A.J. Wilson, V. & Blanchestot, A. *EMBO J.***3**, 439 (1984).
8. Bell, G.I. *et al.* Nature **295**, 31 (1982).
9. Goodbourn, S.E. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 5022 (1983).
10. Yang, T. *et al. Proc, natn. Acad. Sci. U.S.A.* **79**, 6593 (1982).
11. Jabs, E. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4884 (1984).

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100 Years Ago **The Maxim Gun** **From *Nature* 31 414, 5 March 1885.**



MR. HIRAM STEVENS MAXIM, the well-known American engineer, has lately brought out a new form of a machine-gun, which is attracting a great deal of attention in military and naval circles. This gun is a completely new departure. It takes the cartridges out of the box in which they were originally packed, puts them into the barrel, fires them, and expels the empty cartridges, using, for this purpose, energy derived from the recoil of the barrel.

Lysosomes and prohormone activation

SIR – The interesting *News and Views* article by Pfeffer and Ullrich[1] refers to the unexpected relationship between the structure of the low-density lipoprotein and epidermal growth factor (EGF) receptors and that of the biosynthetic precursor for EGF itself. Pfeffer and Ullrich believe that a new clue to the puzzle is provided by the letter in the same issue of *Nature*[2] reporting the detection of unprocessed EGF precursor, but not EGF itself, in mouse kidney. The authors of this letter envisaged the possibility that the EGF precursor is a membrane protein and that it might function as a receptor in the cells of the distal tubules to regulate membrane transport events. This exciting new information, together with other clues to the puzzle, invites the re-evaluation of a suggestion I made a few years ago, prompted at that time by the related problem of the existence of prohormones[3,4].

In brief, it was proposed that polypeptide hormones have evolved from the constituent proteins of the secondary lysosome of simple unicellular organisms. A key element of this hypothesis was that the membrane of the secondary lysosome must contain transport processes. Hence the agents and products of lysosomal digestion would have direct access to, and the possibility of regulating, nutrient transport processes. Regulation of nutrition is a major feature of the action of many polypeptide hormones and growth factors. (As a result of this concept a glucose transport system was searched for and found in lysosomes[5].) It was also proposed that the proteins which could act as polypeptide hormone precursors included membrane transport proteins and lysosomal digestive enzymes. "If some of the effectors arise by partial degradation of membrane proteins, then a structural relationship could exist between some effectors and receptors"[3]. A structural relationship between cobalamin binding proteins and their receptors has been recognized[6].

Further informative structural relationships between a membrane protein, transport, nutrition and growth promoting activity, have emerged around transferrin and its receptor. The transferrin receptor shows weak homologies with the EGF precursor and chicken transferrin precursor[7]. Transferrin itself appears to be structurally and functionally related to an iron-binding cell surface glycoprotein which is present in most human melanomas, fetal intestine and, in trace amounts, normal adult tissues. This protein may translocate iron[8]. Transferrin has growth factor activity and stimulates the growth of some cells independently of its iron binding[9]. Finally the nucleotide sequence of a transforming gene suggests that it encodes a protein partially homologous to the amino terminus of transferrin, suggesting possible functional similarities[10]. It is possible that this smaller peptide is growth-stimulating with a role similar to that proposed for platelet-derived growth factor in malignant transformation[10,11].

Pfeffer and Ullrich also mention that the precursor for a polypeptide homologous to EGF, transforming growth factor (TGF)-alpha, has a sequence suggestive of its being a membrane protein. It should perhaps be added in the present context that TGF-α and EGF show homology to the serine proteases urokinase and tissue-type plasminogen activator[12], and that EGF appears to be related to pancreatic secretory trypsin inhibitor[13]. The EGF precursor also appears to be structurally related to the serine protease factor X and more generally to the family of blood coagulation zymogens which includes prothrombin[14]. Thrombin itself has mitogenic activity and triggers a number of platelet reactions including secretion. These effects are not due to its protease activity but are considered to be hormone-like[15]. Other thrombin sequence homologies which have been described include those with angiotensin and the β-chain of luteinizing hormone[16]. It has recently been recognized that human γ-trace, a polypeptide having some homology with glucagon and corticotropin[17], is an extremely potent cysteine-proteinase inhibitor[18]. The K_i for γ-trace inhibition of cathepsin L is less than 0.005 nM[18]. Thus a picture of close interrelations between some polypeptide hormones, proteases and their inhibitors emerges.

The sequence information which has emerged to date is consistent with the hypothesis that the lysosomes are involved in the activation of prohormones, suggesting that it may provide a useful framework for making and interpreting structural comparisons in this area.

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1. Pfeffer, S. & Ullrich, A. *Nature* **313**, 184 (1985).
2. Rall, L.B. *et al. Nature* **313**, 228 (1985).
3. Hales, C.N. *FEBS Lett.* **94**, 10 (1978).
4. Hales, C.N. & Docherty, K. in *Proteases and Hormones* (ed. Agarwal, M.K.) 19 (North-Holland, Amsterdam, 1979).
5. Maguire, G.A., Docherty, K. & Hales, C.N. *Biochem. J.* **212**, 211 (1983).
6. Grasbeck, R. & Kouvonen, I. *Trends biochem. Sci.* **8**, 203 (1983).
7. Schneider, C. *et al. Nature* **311**, 675 (1984).
8. Brown, J.P. *et al. Nature* **296**, 171 (1982).
9. Tormey, D.C., Imrie, R.C. & Mueller, G.C. *Expl Cell. Res.* **74**, 163 (1972).
10. Goubin, G. *et al. Nature* **302**, 114 (1983).
11. Waterfield, M.D. *et al. Nature* **304**, 35 (1983).
12. Derynck, C. *et al. Cell* **38**, 287 (1984).
13. Hunt, L.T., Barker, W.C. & Dayhoff, M.O. *Biochem. biophys. Res. Commun.* **60**, 1020 (1974).
14. Doolittle, R.F., Feng, D.F. & Johnson, M.S. *Nature* **307**, 558 (1984).
15. Majerus, P.W., Tollefsen, D.M. & Shuman, M.A. in *Platelets in Biology and Pathology* (ed. Gordon, J.L.) 241 (North-Holland, Amsterdam, 1976).
16. Magnuson, S. *et al.* in *Proteolysis and Physiological Regulation* (eds Ribbons, D.W. & Brew, H.) 203 (Academic, New York, 1976).
17. Grubb, A. & Lofberg, H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3024 (1982).
18. Barrett, A.J., Davies, M.E. & Grubb, A. *Biochem. biophys. Res. Commun.* **120**, 631 (1984).

Agreement between optical and radiocarbon dating

SIR — D.J. Huntley *et al.*[1] recently described a new method for dating sediments. They compared their optically dated age of 62±8 kyr for a sample with a radiocarbon dated age of 58.8±0.3 kyr. In fact, the agreement is even better than Huntley *et al.* indicated, since the radiocarbon age used for comparison is based upon the wrong half-life for ^{14}C.

For well over twenty years, it has been internationally agreed to continue to use the Libby half-life value of 5,568 years for ^{14}C (ref. 2), although the best value was acknowledged to be a factor of 1.03 larger[3]. The procedure was justified by the desire to avoid the confusion which would arise should the many thousands of published dates require revision[4]. It should be noted that all radiocarbon dates are consistent among themselves but 3% lower than the true age.

As a result, the true (radiocarbon) age for Huntley *et al.*'s specimen would become 60.6±0.3 kyr, which is in excellent agreement with their 62 kyr value.

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1. Huntley, J.J., Godfrey-Smith, D.I. & Thewalt, M.L.M. *Nature* **313**, 105-107 (1985).
2. *Radiocarbon Dating* 2nd edn (University of Chicago Press, 1955).
3. *Nature* **195**, 984 (1962).
4. *Science* **149**, 1326 (1965).

Right-handed flagella in tumbling *Caulobacter*

SIR — In a pair of fascinating *News and Views* articles, Michael Spencer has recently discussed the remarkable case of bacteria that have right-handed helical flagella but are still able to reverse direction without tumbling, by simply changing the sense of rotation from clockwise to counterclockwise[1]. There seems some uncertainty, however, about when such bacteria were discovered. Spencer presents data from the work of Alam and Oesterhelt on *Halobacterium* species[2]. But the first bacteria to be discovered with such properties were in fact *Caulobacter crescentus* — they were studied by us in the University of Tokyo and the work was reported in an earlier edition of the same journal[3].

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1. Spencer, M. *Nature* **310**, 367 (1984); **313**; 183 (1985).
2. Alam & Oesterhelt *J. molec. Biol.* **176**, 459 (1984).
3. Koyasu, S. & Shirakihara, Y. *J. molec. Biol.* **173**, 125 (1984).

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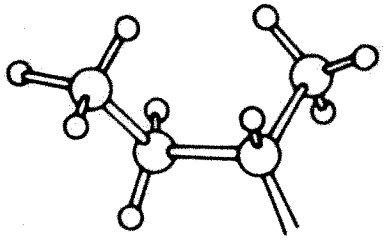
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A tax not for imposition

On Budget day, 19 March, the British government may announce it is to impose value added tax on publications. The proposal might be economically rational but should be resisted.

FOR more than the past six months, British publishers and the British consumers of their products have been buzzing with anxiety at the proposal that there should be a tax on publications. The issue is simple. Should the government, in pursuit of its laudable ambition to shift the burden of taxation from income taxes to what is called indirect taxation (which applies to what people purchase), impose a kind of generalized expenditure tax on publications?

Such a move would be widely and fiercely scorned. On 19 March, when the budget for the coming financial year is known, it should be clear whether the British government is ready to go down yet another unpopular road.

That is why it is important not to exaggerate the case against a tax on publications. Value added tax (known as VAT) is in principle an economically rational and an equitable form of tax. The idea is that, in the manufacture of goods or the provision of services, each commercial entity will pay VAT on all purchases used in its business, collect VAT from all its customers, and hand over the difference between the output and the input tax to the government. At the end of each commercial chain stands the ultimate consumer, for whom VAT is a tax on expenditure. But because of the way in which the tax is structured, the burden is collected from all who have contributed to the production of something saleable in proportion to the value they have added to the final product. By now, VAT is a familiar part of the European scene, and even the basis on which the annual budget of the European Communities is calculated. As taxes go, VAT is a splendid principle. It would no doubt be more popular if it were more easily understood, and if its administration were not appallingly cumbersome.

In parochial Britain, for the past six months the popular slogan thrown at the proposal to levy VAT on publications is that such a move would be a "tax on knowledge", but that is not easily sustained. The most obvious difficulty is that there are many types of publication which can hardly be called educational or scholarly. Further, there are already many intellectual commodities in Britain and elsewhere in Western Europe which are taxed in exactly the same way. Those who buy tickets at theatres, opera houses and concert halls are already paying VAT. Who is to say that these institutions contribute nothing to the pool of knowledge of cultivated societies? That a performance of Beethoven's *Ninth Symphony* should be taxed when *Pride and Prejudice* is exempt?

As things are, in Western Europe, something like 40 per cent of purchases of goods and services is subject to VAT, in most countries at a single percentage rate (which is, at present, 15 per cent in Britain). VAT is not levied on necessities of life such as food (except in restaurants) and rent, but unambiguously attaches to most manufactured goods, television sets for example. Publications are not taxed in this way, while commercial publishers are "zero-rated" in the sense that they neither pay tax on their inputs nor charge it on their products. Elsewhere in the European Community, in West Germany and, more recently, in Ireland, VAT applies.

So on what grounds should publications continue to be lumped in with food (but not drink) as necessities of life? The easy argument, that a tax that impedes the free flow of information within a democratic society is a threat to its well-being, is too riddled with inconsistency to be easily defended. One difficulty is that it has been readily, perhaps too readily, accepted that the commercial organizations willing to provide cable television services in Britain (and still waiting to see whether there is substance in the government's promise to "cable Britain") will be taxed by VAT. Another is that telephone calls, which are as powerful a means of conveying information as many publications, are also taxed, perhaps iniquitously. Compliantly to have accepted these taxes but to resist that now proposed for printed publications is inconsistent and, therefore, unwise.

The true case against the British government's flirtation with the proposal to levy VAT on printed publications is different and more pragmatic. There is a host of ways in which VAT would have disastrous consequences, one of which should be clear from the pages which follow where the virtues of a year's crop of textbooks are reviewed. A glance at the prices shown at the head of each review will be to most people a prompt for the question "how can students afford them?". So how will they get by if the cost is increased by another 15 per cent? Further depriving them of essential tools would throw doubt on the quality of education as such.

The case of learned journals is more complicated, and more shot through with potential inequity. The National Book Committee in Britain, an admitted lobbyist against the VAT proposal, has nevertheless produced some telling figures as part of its case. Surprisingly, it calculates that learned journals produced in Britain account for some 28 per cent of the titles in international currency, but that the sales of these journals within the United Kingdom account for only 21 per cent of the total (reckoned as £187 million a year).

Even allowing for what may be very large errors in these estimates (based on replies to questionnaires), the figures suggest that the plan to levy VAT on publications would be a way of further and arbitrarily increasing costs in a modestly substantial export business where price-resistance is already strong. (Libraries everywhere are short of funds.)

The way in which the effects of VAT would discriminate between different kinds of customers and publishers would however be absurd. Commercial publishers (such as those of *Nature*) would at least be able to offset their input tax (paid out) against their output tax (collected from customers), but learned societies would have no such relief. Similarly, universities (already skimping unwisely on library acquisitions) would have no way of recovering the tax paid to publishers, but the libraries of commercial research laboratories, for example, would be able to deduct VAT paid on journals from that collected from the ultimate customers of their operations.

And while, in theory, VAT should be levied on imported publications, there is plainly no way in which this could be done on subscriptions serviced from abroad. (As Ireland has discovered to the cost of its own newspapers; VAT has been a boon to those who ship in British newspapers.) Yet a little discouragement of this kind could provoke a rapid emigration of many important journals that could be based anywhere in the world, and which are in Britain by historical accident. Is that what the British government wants to see occur?

But why not anticipate these untoward consequences of the imposition of VAT, and make arrangements that vulnerable groups of users, or publications, should be exempt? That seductive argument is the most dangerous of all, for it is a way of inviting the British government to discriminate between different kinds of publications. It amounts to censorship of a kind. Nobody, perhaps not even the government, wants that.

This is why a sensible decision about VAT on publications is bound to be a difficult decision. The government may like to think that the benefits of less direct taxation will put so much more money into people's pockets that the obvious economic consequences of the tax will be outweighed. In a more prosperous Britain, there might be something in that calculation. But as things are, VAT would further undermine the British academic enterprise, would begin a flight from Britain of many important institutions and would spread yet more intellectual impoverishment in a society that has long since lost its reputation for being widely read. Living with a little inconsistency is the better course. □

Matters of gravity

John D. Barrow

General Relativity and Relativistic Astrophysics. By Norbert Straumann.
Springer-Verlag: 1984. Pp.459. DM 112, $42.
A First Course in General Relativity. By Bernard Schutz.
Cambridge University Press: 1985. Pp.376. Hbk £30, $54.50; pbk £9.95, $19.95.
General Relativity. By Robert M. Wald.
University of Chicago Press: 1984. Pp.491. Pbk $34, £31.50.

EVER since the classic texts by Weyl and Eddington, written soon after the inception of general relativity, there has been a steady stream of high quality expositions of Einstein's route to his new theory of gravitation. Most have stayed close to the path first beaten by Einstein himself, which he described in the many editions of his own *The Meaning of Relativity*. Subsequent descriptions of general relativity have tended to focus upon polishing-up the standard route towards the field equations taken by Einstein, expounding the equivalence principle, covariance, tensor analysis, curvature and the field equations, before moving on to Schwarzschild's solution and the classical experimental tests of the theory. The situation is slightly reminiscent of the multitude of users' guides for the better known personal computers, each a minor revision of the manufacturer's manual.

Yet for all this duplication the didactic profile of general relativity has been dominated by a small number of influential books with very definite, but very different, points of view as to which aesthetic criteria should be emphasized in its formulation. While some authors regard the geometrical basis of the theory as the essential element in its formulation, others see this feature as an occlusion that inhibits its connection with the rest of physics. While one author may take a version of the principle of general covariance as its paramount guiding light, another may ignore it entirely or stress that it is empty of all physical content. This essential ambiguity in the initial motivation for entering into the mathematics along the lines followed by Einstein was the main reason for the proliferation of textbooks until the early 1970s. Since then, the motivation has changed: the development of more elegant and powerful coordinate-free formalisms has dominated the modern research literature in relativity and has begun to be promoted in new teaching texts. Further, there have been exciting new applications of general relativity to astrophysics and in the development of a unified approach to the natural forces. General relativity has finally ceased to be embarrassingly disjoint from the rest of physics, a haven only for group theorists and differential geometers.

These three books reflect the need for systematic expository texts that treat new theoretical and observational developments in modern mathematical language. Straumann's volume is the most formal in style, and has arisen from series of graduate lectures. It is divided into three parts which hang rather uneasily together.

The first part is a rigorous and detailed description of modern differential geometry and could be used as a course on this topic. Very clearly written, it takes the approach of the mathematician rather than that of the physicist; beginning with differentiable manifolds, it leads on to differential forms and the use of coordinate-free techniques for describing curvature. The second part then develops general relativity and contains detailed chapters on the behaviour of weak gravitational fields and the post-Newtonian approximation. Here, I found the exposition clear when the emphasis was on the mathematical formalism but rather weak on the link between physical principles and the mathematics. The last third of the book is much more physical and deals with relativistic astrophysics: neutron stars, the Kerr black hole and the laws of black hole mechanics, binary X-ray sources and, in the final chapter, the problems of accretion disks around black holes. All these astrophysical chapters are written at a high level but fall short of the standards set by Shapiro and Teukolsky's outstanding graduate textbook, *Black Holes, White Dwarfs, and Neutron Stars* (Wiley, 1983). There are a small number of exercises scattered throughout the text but I did feel this to be an intended textbook that was written and printed in the somewhat forbidding style of a monograph.

Schutz is a well-known relativist who specializes in the study of gravitational radiation and equations of motion in general relativity. In this book he has put together a very carefully constructed course on general relativity which, unlike Straumann's, can be used by both undergraduates and graduates. Its great strength is the author's evident experience in teaching its content. The book is a goldmine of cleverly constructed problems and exercises (and solutions!) — there must be nearly 500 in total and a lot of careful planning has gone into their devising. This feature alone is sufficient to make this text far more appealing than Straumann's.

Schutz devotes the first 50 pages to recasting special relativity and vector analysis in modern formalism, before launching into tensors. Yet one wonders whether the level of detail here might not inhibit all but those able to chart a short-cut through this material from employing it as a core text, simply because of the limited length of undergraduate courses at British universities. But those seeking a basis for an advanced graduate course on the behaviour of gravitational radiation and black holes will find the author's evident mastery of the subject, and his ability to avoid mathematical formalism for its own sake, a strong attraction. The final chapter gives a brief but useful introduction to the homogeneous and isotropic cosmological models. Strongly recommended.

Wald's book is for a graduate course but, unlike its two competitors here, will be also extensively used by research workers because it is the first pedagogic treatment of so many advanced topics and techniques that form the research frontier of the subject. The author employs the so-called



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abstract index notation rather than traditional tensor notation. The formal treatment of manifolds, differential forms, continuous transformation groups, conformal transformations and the Hamiltonian formulation are all contained in extensive appendices that are designed to be integral parts of the text, displaced from the main body only on the grounds of continuity.

The first portion of the book covers the traditional ground from special relativity to Einstein's equations in a mathematical manner that I found was slightly lacking in its discussion of the physical motivation and content of the formalism; perhaps it is assumed that the reader has already followed a traditional introductory course? However, the outstanding part of this book is the treatment of a wide range of advanced topics in the second half of the text. Modern techniques for generating and classifying exact solutions to Einstein's equations, the causal structure of space-time and the use of techniques from differential topology to prove singularity theorems are all developed in detail. Each chapter is followed by a number of problems, although solutions are not supplied. The final series of chapters provides modern discussions of the initial value problem and Penrose's conformal treatment of infinity. These later chapters also focus upon topics close to the author's own research interests and there is a particularly nice introduction to developments in quantum gravity and the study of quantum fields in a classical curved space-time as well as detailed discussion of black hole mechanics and thermodynamics.

This is a much-needed book that will greatly stimulate the teaching of advanced graduate courses in modern gravitation physics. It will also provide an invaluable directory of modern techniques and results for gravitation theorists. □

John D. Barrow is a Lecturer in Astronomy at the University of Sussex.

Physical reasoning

Colin Upstill

Theoretical Concepts in Physics.
By M.S. Longair.
Cambridge University Press: 1984. Pp.366. Hbk £25, $49.50; pbk £8.95, $14.95.

IT IS the exception rather than the rule for brilliant scientists to be the best teachers: Planck studied under Helmholtz and Kirchoff, but confessed that "the lectures of these men netted me no perceptible gain". Most of us have at some time had the same experience, going full of expectation to a lecture by some professor of renown, only to find the lecture-room clock of more interest than his impenetrable phrases and even more impenetrable algebra.

Sadly, good textbooks are a rarity too. The texts whose content best matches our syllabuses are all too often hastily compiled pot-boilers, spin-offs from others' fumbling attempts at courses with similar titles to our own. It is of the greatest value in university teaching to possess a full and deep understanding of one's subject matter, yet to be aware of what it is like to be a student who does not. Professor Longair has this rare gift, and he has used it to good effect in writing this excellent book, subtitled *An Alternative View of Theoretical Reasoning in Physics for Final Year Undergraduates*. The subtitle hardly does the book justice; it is a complement rather than an alternative to most standard courses and standard texts — providing the cement of understanding to put between the blocks of lectures which go to build a physics course.

For most of the topics covered, Longair has leant heavily on the best treatments available elsewhere, and has often gone back to the original literature — for men such as Einstein, Maxwell and Rayleigh expressed lucidly ideas which subsequent generations of authors have muddled and obscured. It is no surprise to find that the book owes a great debt to Feynman's three-volume *Lectures on Physics* (Addison-Wesley, 1963–1965). But let me make it quite clear that Longair's book is much more than a collation of familiar material from diverse sources; throughout, it is stamped indelibly with the author's own deep insights and, equally important, his enthusiasm for his subject. This enthusiasm comes over vividly, and has resulted in a learned book with the rare property that it is compelling: like a well-crafted novel, it is hard to put down; at the end of each chapter, one turns the page eager to see what happens next. In my view, Longair has provided for final-year physics undergraduates the sort of essential background Feynman gives them in their first and second years. No facet of the reality of the practice of physics is omitted, from the importance of experimental observations for theory (via Kepler's use of Tycho Brahe's observations and especially his error analysis, which was so crucial to the discovery that the orbits of the planets must be elliptical rather than circular), through the use of models (such as Maxwell's mechanistic models for electromagnetic phenomena), to the use of computers (to simulate the synthesis of light elements in the hot big bang).

In his epilogue, Longair admits that in preparing the book he learned a great deal which he wished he had known years ago. I feel the same after reading it. □

Colin Upstill is a B.P. Venture Research Fellow at the H.H. Wills Physics Laboratory, University of Bristol.

Round the mechanics course

J.W. Humberston

Classical Mechanics*.
By B.P. Cowan.
Routledge & Kegan Paul: 1984. Pp.111. Pbk £3.95, $9.95.

CLASSICAL mechanics continues to be regarded as an essential component of a modern degree course in physics, and this book attempts to cover all the material required by a student taking a first course in the subject. It is a rather bold claim to make of a book containing only 111 pages, but the choice of topics is generally satisfactory. There are chapters on Newton's laws, energy and momentum, dynamics of a single particle, many-particle systems and rigid bodies, and non-inertial frames. The final chapter gives a very clear introduction to Lagrangian and Hamiltonian mechanics, ending with a brief section on Poisson brackets and their relationship to commutators in quantum mechanics. Worked examples are distributed throughout the book and a short set of problems (with solutions) is also included.

Because the book is so small, there are, inevitably, omissions. For me, the most serious of these relates to moments of inertia. After a brief account of the inertia tensor there is merely the statement that the diagonal elements are the moments of inertia. No mention is made of the motion of a rigid body about a fixed axis (unless it be a principal axis) nor of the theorems of parallel and perpendicular axes. There is not even a worked example of the calculation of a moment of inertia.

Also, in the discussion of motion under an inverse square law of force, insufficient detail is given of the properties of conic sections. It is assumed, I would have thought unreasonably, that the reader is already familiar with them. A bibliography of more extensive works in mechanics would have been some compensation for these and other omissions but, unfortunately, one is not provided.

The book is written in a clear and concise style and, despite its shortcomings, should generally be well received by its intended readership. □

J.W. Humberston is a Lecturer in the Department of Physics and Astronomy at University College London.

*A volume in Routledge & Kegan Paul's *Student Physics* series. Other titles to appear during 1984 were *Quantum Mechanics* (by Paul Davies), *Relativity Physics* (Roy Turner) and *Electricity and Magnetism* (Roland Dobbs). All are short paperbacks costing £3.95, $9.95. Forthcoming later this year are *Electromagnetic Waves* (Roland Dobbs) and *Liquids and Solids* (Michael Sprackling).

Good companions

Robert Connon Smith

Astrophysics. Vol. I Stars. Vol. II Interstellar Matter and Galaxies.
By Richard Bowers and Terry Deeming.
Jones and Bartlett/Wadsworth: 1984. Vol. I pp.344, Vol. II pp.244. $37.50, £17.95 each volume.

THE main teaching load for astronomers in the United States is the large first-year class of liberal arts students and so there are many textbooks jostling for a place in that market. In British universities it is more common to teach astronomy only to science specialists, usually in the context of physics courses. Good books at that level are rarer and so it is a pleasure to find a new two-volume work that caters for the advanced undergraduate or beginning graduate student.

In 588 pages the books attempt to cover the whole of modern astrophysics, excluding the Solar System. The task is made easier by the assumption that the reader already has an elementary knowledge of astronomy, presumably from one of the myriad first-year descriptive texts, but nonetheless the authors succeed remarkably well in providing a comprehensive coverage at a useful depth. They are not content with order-of-magnitude arguments — though these are well-used where appropriate — but give proper mathematical derivations while managing to avoid the generality of treatment that only serves to confuse the new student.

This is not a research monograph and so is not completely up to date. For example, the discussion of supernova models mentions radioactive decay as an explanation for the exponential tail in the light curve of a Type I supernova but attributes the decay to Cf^{254} rather than to the more likely Co^{56}. In the more general discussion of stellar evolution, there is little emphasis on mass loss, except through mass transfer in binary systems. However, the volume on stars is more complete than its companion, in which there is little hint of the modern picture of the violent interstellar medium, and almost no discussion of interstellar molecules. The exciting subject of active galactic nuclei and quasars gets essentially no mention at all. These and other omissions make the books more suitable for undergraduates than for graduates, though the latter will learn useful things about longer-established branches of astrophysics. Even in these branches there are some surprising gaps, however, such as the absence of any detailed account of how the rotation curve near the Sun is found. As a whole the second volume is not as good as the first, being less complete and less balanced. It also stops rather abruptly, with no attempt to draw ideas together.

Still, I liked these books. They are readable and well-illustrated, full of short but useful problems and include some helpful suggestions for further reading. Without producing something twice the length it is doubtful if a much better job could have been done, and I shall certainly be including them on reading lists for my own students. □

Robert Connon Smith is a Lecturer in Astronomy at the University of Sussex.

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Out of this world

David W. Hughes

The Universe of Galaxies: Readings from Scientific American.
Compiled by Paul W. Hodge.
W.H. Freeman: 1984. Pp.113. Hbk $20.95, £23.95; pbk $10.95, £12.50.
A Workbook for Astronomy.
By Jerry Waxman.
Cambridge University Press: 1985. Pp.356. Comb bound £15, $19.95.
Planets and Their Atmospheres: Origin and Evolution.
By John S. Lewis and Ronald G. Prinn.
Academic: 1984. Pp.470. Hbk $59, £49.50; pbk $29.50, £25.
The Solar System.
By Barrie William Jones.
Pergamon: 1984. Pp.336. Hbk £24.25, $40; pbk £11.75, $19.50.

ASTRONOMERS at the chalk face of tertiary education have a lot to be grateful for, not the least being the hard work of authors and publishers. Astronomy has popular appeal, and so attracts the attention of glossy scientific journalism; it has a strong practical edge and many new ideas for laboratory experiments have been laboriously written up into book form; and it is fast moving, an attribute that results in a stream of updated textbooks.

At the forefront of the popularization of astronomy are the monthly astronomical reviews that appear in *Scientific American*, noteworthy for their clarity of exposition — both in text and in figures — and the erudition of their authors. Periodically the publishers gather together articles on a specific topic and issue them in a large-format paperback. *The Universe of Galaxies* is one such collection. It starts with a paper by Bok on our own galaxy and moves out, encompassing the Andromeda galaxy, dark matter in spiral galaxies, the evolution of disk galaxies, tides between galaxies, active galaxies, cosmic jets, superclusters, voids and quasars. The important word in the title of the book is *Readings*. It has never been easy getting students to read and *Scientific American* must be praised for coaxing many and enlightening most.

Practical work is crucial to astronomy — observations must be made, spectra analysed, graphs plotted, errors calculated and results mused over. But how do we introduce this aspect of the subject to the throngs in arts faculties that take a science course in astronomy at American universities. These students dive into astronomical texts by the likes of Abell, Pasachoff and Hartmann and read, somewhat grudgingly, enough of them to pass the course. They must be encouraged to wander into the laboratory planetarium and observatory. Waxman's book is a valuable start, providing a complete set of practical activities to supplement the factual meal to be gleaned from the textbooks. Suggested activities are divided into three time categories, short ones which can supplement a lecture, longer ones suitable for a three-hour laboratory class and others concentrating on outside observations which can be carried out individually and can extend over many weeks.

A Workbook for Astronomy contains a multitude of bright ideas and, what is more important, the wherewithal for carrying out the experiments — finder charts for binaries, comparison spectra, lunar charts, reproductions of crater fields, star charts and photographs of clusters of galaxies. Students are introduced to a wealth of topics including stellar evolution, distance measuring, planetary orbits, the curvature of space, the age of clusters and classification of galaxies. They learn by doing. I found the book a source of many good ideas but for British science teaching the standard is rather elementary. Also,

while I can see very little advantage to the student in having the book held together by a plastic ringbinder, the advantage to the publisher is obviously enormous. After one year of use the book falls to pieces and the owner cannot recoup some of his outlay by selling it to those who follow. The quest to reduce the second-hand value is also helped by including "data sheets" and graph paper (with marked axes!) in the text. This surely spoonfeeds students too much, for one of the objectives of laboratory exercises is to introduce them to the craft of tabulating results and planning graphs.

The other two books reviewed here both deal with our Solar System. Lewis and Prinn have provided, in *Planets and Their Atmospheres*, the ideal starter pack. It is not for the faint hearted, however, being aimed accurately at the final-year student, postgraduate and research worker. To this end the authors have assembled an impressive 70 pages of references and a detailed, itemized index which has at most five page numbers for each topic. The book is also timely. Spacecraft have provided an enormous bank of data about the present atmospheres of the terrestrial planets Venus, Earth and Mars. Observations of Jupiter, Saturn, Uranus, Neptune, Titan and Io have also helped us formulate models for the structure, composition and chemistry of their gaseous mantles. Detailed comparative studies have allowed us to move away from the present state to a discussion of origins and evolution.

Why do I call it a "starter pack"? Well, when considering a new topic, all researchers and students need to read a few sections of a good book to familiarize themselves with the relevant background before turning to shelves of journals in the library. There are too few good books of this type around. We must be grateful to Lewis and Prinn for providing another.

Jones's *The Solar System* is a non-mathematical introduction to our planets. I found it rather difficult to visualize the audience it is intended for. We are given a contemporary picture of the planets, with many references to the new data obtained from spacecraft, but the view has been somewhat drained of its excitement. Everything is mentioned, but only briefly and sketchily, and the author has thus given himself little room to point out the problems and mysteries. He has also taken the rather old-fashioned approach of compartmentalizing each planet into a separate chapter, which militates against fruitful comparisons. And now that we are so used to pages of colour images of planets, the reversion to black-and-white produces a sense of disappointment.

There are many general books presently available which deal with the Solar System. Unfortunately, most of them are better than this one. □

David W. Hughes is Senior Lecturer in Astronomy and Physics at the University of Sheffield.

From eye to CCD

Michael Rowan-Robinson

Astrophysical Techniques.
By C.R. Kitchin.
Adam Hilger: 1984. Pp.438. Hbk £40, $65; pbk £15, $25.

IN THE 50 years since Jansky first detected the Milky Way at radio wavelengths there has been a phenomenal explosion in techniques of astronomical observation. The new wavelengths — radio, X-ray, ultraviolet, infrared, microwave, gamma-ray — and the other types of astronomical information reaching the Earth, such as neutrinos, cosmic rays and gravitational radiation, have each required new detection techniques, new types of telescope and new observing platforms, ranging from caverns deep under the Earth to satellite-borne telescopes. Optical astronomy too is being revitalized by new techniques, for example the CCD (charge-coupling device) array and the fibre-optics spectroscope.

Many university and polytechnic departments of physics or astronomy give courses on astronomical observing techniques and instrumentation. Not all of these bear much relation to what is actually happening in astronomy today, and there is a great need for authoritative and accessible books on the subject. Dr Kitchin has set out to provide an account suitable for the science-based undergraduate, though he hopes that the amateur astronomer will find it useful and also points out that increasing specialization in the subject means that most professional astronomers would also benefit from such a book.

Dr Kitchin concentrates on techniques of detection and imaging, but extends this to include the basic optics used in modern telescopes. There is little discussion of the atmospheric and other background limitations which pose such a challenge to observational astronomy and which drive so many of the technical developments. The book is crammed with useful detail, though. It is strongest on the techniques of optical observation: the coverage of the new astronomies such as radio and X-ray is fairly skeletal. Microwave and submillimetre developments are not mentioned. The physical basis of the techniques is briefly discussed, but a fair knowledge of atomic physics and optics is implicitly assumed.

This will be a useful addition to the reading list for a more advanced course on astronomical techniques. But the amateur astronomer may find it a bit heavy going.

Michael Rowan-Robinson is Reader in Astronomy at Queen Mary College, University of London.

Inside the planets

D.C. Tozer

Physics of Planetary Interiors.
By G.H.A. Cole.
Adam Hilger: 1984. Pp.208. Hbk £22, $39; pbk £9.95, $16.

THERE will always be a need for books that try to put the enormous complexity of the known planetary structure and behaviour into a readily comprehended form — books that show a student how much can be explained by a relatively straightforward application of generally accepted physical principles. Professor Cole's book is directed at undergraduates and his choice of subject and approach reflect his background in applied mathematics. For example, much — in my view, too much — attention is given in this introductory text to the gravitational fields and shapes of rotating massive bodies in hydrostatic equilibrium (though who would now deny that a world of meaning and whole fields of research have been inspired by the very small but definite departures of the actual geopotential surfaces from such predicted shapes?). A student needs much more guidance than he is given here for pursuing calculations that tacitly imply a very static view of planetary evolution.

Characteristically, an attempt is made to define the realm of planetary science as that of objects in which self-gravitation is strong enough to induce an axial symmetry of their external shape, but not so strong as to make the atoms "incapable of maintaining the equilibrium which defines a planetary body". Such efforts at classification appeal to the tidy mind, but are likely to prove very confusing to the able and inquisitive student. "Minor planet" is a well-established usage for objects whose light curve suggests a highly irregular shape, and if the quotation I have taken is intended to mean no pressure ionization of atoms, what is one to call an object such as Jupiter, in which a substantial mass fraction is believed to be a pressure-induced metallic phase? Further clarification is not helped by the erroneous comment that free electrons are non-relativistic in planets but fully relativistic in white dwarf stars.

Unfortunately, this kind of ambiguous or poorly reasoned remark is all too common — I noticed serious conceptual errors and typographical mistakes that make the sections on planetary heat transfer and magnetism positively misleading. If there is no treatment of seismology, a chapter devoted to a hydrostatic theory of the structure of icy major planet satellites is an original feature. Nevertheless, I would not recommend this book to students until several corrections have been made. □

D.C. Tozer is a Reader in Theoretical Geophysics at the University of Newcastle upon Tyne.

Eumorphous amorphs

Robert W. Cahn

Physics of Amorphous Materials.
By S.R. Elliott.
Longman: 1984. Pp.386. £25, $60.

ONE of the joys of contemplating amorphous materials is their affinity with paradox. *Amorphous*, etymologically, signifies *shapeless, without form*; yet the book under review has an 80-page chapter on structure. One of the key theorems general to all amorphous solids is the Kauzmann Paradox, which tells us that for all glasses (a concept not quite coterminous with amorphous solid) there is a temperature above 0K at which the entropy must decrease below that of a crystal of the same composition; Dr Elliott offers a careful discussion of the implications of this paradox, which belongs to the category of impossible things that Lewis Carroll's White Queen liked to believe before breakfast.

Again, Dr Elliott repeatedly insists that an amorphous material has no reciprocal lattice, and this poses enjoyable obstacles to theorists who seek to understand, in particular, electron transport in these materials. But even this "reciprocity failure" may not be a universal attribute of amorphous materials, as witness the publication, a few weeks ago, by Shechtman and others, of evidence of a non-periodic metallic phase (a quasicrystal), neither glass nor crystal, which yet gives a sharp diffraction pattern... of fivefold symmetry!

There is a plethora of textbooks and monographs on glasses, but until recently the word has tacitly connoted oxide glasses. A leading exemplar of this large category is *Glass Science* by R.H. Doremus, published in 1973, which is entirely about oxide glasses, an excellent book, subject to that limitation. J. Zarzycki's *Les Verres et l'Etat Vitreux* (1982) takes this classic approach to glass science as far as it will go. Lately, a flood of literature, but no textbooks, about metallic glasses has appeared. Other textbooks again, notably Mott and Davis's celebrated *Electronic Processes in Non-Crystalline Materials*, have covered the theoretical physics of conduction in chalcogenide glasses.

Elliott approaches the field in a new way. He treats amorphous materials quite generally in terms of preparation strategies, ease of glass formation, the glass transition and its statistical mechanics and thermodynamics, and, in particular, structure. That chapter has the clearest exposition of diffuse diffraction from glasses and of EXAFS that I have seen. Defects are also discussed; of course, a genuinely formless material could not possibly have defects, since the existence of defects implies the existence of a non-defective structure! The author pursues a resolutely quantitative approach but combines it with frequent pauses to see where he has got to in terms of a physical model. The reader is thus helped to keep track of the argument. Problems at the end of each chapter, linked always to the relevant mathematics, will help the conscientious reader to cement his understanding.

The heart of the book is a more specialized theoretical treatment of spectroscopy and electron transport, mostly with reference to semiconducting (chalcogenide) glasses but also to metallic glasses. A number of difficult topics are painstakingly set out; I have never seen such a clear discussion of the difficult concept of two-level systems. However, as the author himself specifies, the reader must be competent in solid-state physics somewhat beyond the level of Kittel's *Introduction to Solid State Physics*, and this distinctly narrows the readership.

This fine book has at present no effective rival. Although — inevitably — it barely touches on some aspects of some types of amorphous solid, it is yet an excellent basis for an advanced course on the amorphous state for physicists. □

Robert W. Cahn is currently Visiting Research Fellow at the General Electric R&D Center, Schenectady, New York.

On the ground

C. Kidson

Geomorphology.
By Richard J. Chorley, Stanley A. Schumm and David E. Sugden.
Methuen: 1984. Pp.605. £17.95, $30.
The Nature of the Environment: An Advanced Physical Geography.
By Andrew Goudie.
Basil Blackwell: 1984. Pp.331. Hbk £22.50, $39.95; pbk £7.50, $14.95.
Environmental Systems: An Introductory Text.
By I.D. White, D.N. Mottershead and S.J. Harrison.
George Allen & Unwin: 1984. Pp.495. Hbk £30, $50; pbk £11.95, $19.95.

MORE than 80 years ago, long before the current environmental movement began, geography was described as "the study of man in his environment". However physical geography, environment and geomorphology, as covered by these three volumes, have differing meanings. There is, nevertheless, sufficient overlap to make it useful to look at them together.

In the narrower field of geomorphology, concerned with the geometry of the surface features of the Earth, the new work by Chorley, Schumm and Sugden must be hailed as a masterly review of the development of the science and of its present status and subject matter. It will rank with the classical presentations of Lobeck (1939) and Von Engeln (1948). Its publication may, indeed, come to be regarded as marking almost as significant a stage in the evolution of the subject as the major works of Davis (1909), Penck (1924) and King (1967). Time will be needed to test this view, which some may regard as premature and perhaps extravagant.

The great merits of the book are its balanced presentation of the different ways of looking at the development of landforms and the integration of the historical and functional approaches. Part 1 represents the clearest, most concise and penetrating introduction to the subject which it has been my pleasure to read. While the whole work is clearly aimed at advanced students and professional geomorphologists, this section can be recommended even to first-year students. This is so even though they may not fully comprehend the whole of it and despite the fact that it does less than justice to the studies of denudation chronology, largely carried out by students of that greatest of English Davisian disciples, S.W. Wooldridge. Part 3 is a comprehensive review of contemporary Earth surface processes which brings the advanced student to the very frontiers of research. The remaining sections on geological (Part 2) and climatic geomorphology (Part 4) are penetrating syntheses of these areas of the subject.

The book as a whole is a balanced statement of the present position in geomorphology. The specialist interests of the authors have been combined in a most successful unified approach, and while it is sometimes possible to detect the authorship of particular sections only those very familiar with their individual work will be able to do so.

It is probably unfair to compare this outstanding volume with the new study of the environment by Professor Goudie, which is sub-titled *An Advanced Physical Geography*. This book covers not only geomorphology but includes also climatic and ecological sections and a consideration of man's impact on the environment. Nevertheless, by far the greater part of the subject matter is the same as in Chorley *et al.* In addition, the publishers claim that Goudie's book "is informed by the latest advances in theory and research" and that it "will surely become the standard work". For these reasons alone comparison becomes inevitable.

It has to be said at once that *The Nature of the Environment* suffers in the comparison. It is written in a discursive and descriptive style, with frequent statements of the obvious such as "The great bulk of the world's water is in the oceans" and "During glacials the ice caps of the world were greatly expanded" which one might expect to find in a first year essay. One example serves to show that the publisher's claim for a research basis is somewhat

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overdrawn. The short and inadequate section on sea level changes shows no acquaintance with the work, between 1974 and 1982, of the International Geologic Correlation Project No.61, on sea level change in the last 15,000 years. Similarly the claim that it will become the standard work looks somewhat exaggerated in the light of a reference list confined solely to textbooks. In short, the book has some merit as an introduction to the subject but is not for advanced students and research workers.

The last of this trilogy, *Environmental Systems*, is a modest approach to the very



U.S. Department of the Interior

Subject for the geomorphologist — the Grand Canyon, drawn c. 1880 by William H. Holmes.

broad subject of "the environment". As an introductory text, which it claims to be, it has much to offer. Unlike Chorley *et al.* the authors do not set out to embrace, or even to reconcile, all approaches to their subject matter but concentrate firmly on the single methodology of their title. In this the book is very largely successful. A careful factual exposition is supported by frequent references to source material, by detailed suggestions for further reading and by a bibliography of 350 journal references. It is a little disappointing that Part C does not include any treatment of coastal environments — the book's aim to promote "an understanding of Man's terrestrial environment" surely cannot be entirely achieved if it ignores a part of it where such a large number of people live and work. Despite this shortcoming this is a successful book and one which can be recommended to the readership at which it aims.

The strength of interest in "the environment" is shown by the steady flow of publications in this general field at all levels. Occasionally an outstanding book in a specialized part of the subject appears and tends to overshadow other worthwhile contributions. *Geomorphology* by Chorley, Schumm and Sugden is such a work. Still, the other volumes reviewed here also have their strengths and will be of value to somewhat different readerships. □

C. Kidson is Emeritus Professor of Physical Geography at the University College of Wales, Aberystwyth.

Devonian review

Bruce Sellwood

Aspects of a Stratigraphic System: The Devonian.
By D.L. Dineley.
Macmillan, London: 1984. Pp.223. Hbk £20; pbk £9.95.

THE Devonian Period spans some 48 million years (408-360 Myr) and was originally established, in Devon, as the Devonian System by the entrepreneurial stratigraphic approach of Sedgwick and Murchison in 1839. David Dineley, who has devoted many years to research on the Devonian and its fish faunas, has written this book to illustrate, he says, the ways in which the different disciplines within geology help our understanding of how things were 360 million years ago. He claims that it is aimed at the "interested" but "not very advanced student of historical geology".

The book doesn't really provide a source on regional Devonian geology (though in fairness one should say that it doesn't set out to do so); thus it cannot be used in the same way as Arkell's masterly descriptive account, *Jurassic Geology of the World* (Oliver and Boyd, 1956). Neither does it provide an ideas approach to a system comparable with that of Hallam's *Jurassic Environments* (Cambridge University Press, 1975). Instead it falls somewhere in between.

The introduction attempts to justify use of the Devonian as a vehicle for the book "because it is there", an argument that also recurs in subsequent chapters on the materials of stratigraphy, and stratigraphy and the world tectonic model. In the latter, plate tectonic models are extensively adopted from the literature, although being considered "crude means of solving problems in crustal history". The next chapter ("Time in Question") shows how stratigraphic correlation is achieved, while Chapter 5 deals with biostratigraphy and reviews the various groups of organisms found in Devonian rocks and their use in correlation.

The following four chapters deal with Devonian facies: Old Red Sandstone; paralic facies (including some carbonates); carbonates and evaporites; and deep-water suites. Each begins with a generalized global review before giving brief accounts of individual areas (Arctic Canada, western Europe, the Russian Platform, Altai-Sayan, east Asia and so on). Many maps are included (though only a single isopachyte map occurs in the whole book), but relatively few stratigraphic sections. This is a pity in view of the fact that, after correlation, successful stratigraphic interpretation rests on the details of sequence evaluation.

Finally, Dineley considers Devonian geography, particularly covering recent work on possible continental dispositions, facies and the oceanographic implications. Climatic changes are reviewed and notions of meteorite impact speculated upon.

I'm unsure about the readership for this book. It cannot really be for "interested" students, because they will get lost in the sections on biostratigraphy; and it won't be for the specialist Devonian workers because this is a broad scale review. Still, it should be taken up by university libraries where it will be a useful student text for the few sessions normally spent on Devonian geology. □

Bruce Sellwood is a Lecturer in Geology at the University of Reading.

Earth through time

A. Hallam

The History of the Earth's Crust.
By Don L. Eicher, A. Lee McAlester and Marcia L. Rottman.
Prentice-Hall: 1984. Pp.197. Hbk $25.60, £23.30; pbk $22.90, £20.85.
The Evolving Continents, 2nd Edn.
By Brian F. Windley.
Wiley: 1984. Pp.399. Hbk £25, $24.95; pbk £9.95, $21.50.

IN THE current enthusiasm to understand more about geological processes in such diverse fields as sedimentation, rock deformation and volcanism, increasing use is made of mathematical modelling and modern laboratory techniques. This analytical approach remains somewhat sterile, however, if it fails to illuminate Earth history, which remains at the core of academic geology. Because the subject is so complex and involves synthesis of a diversity of rapidly growing research fields, satisfactory undergraduate textbooks are few and far between. As in other spheres of science the premium in research is on increased specialization and this inevitably influences what is taught in university courses.

The advent of the new book by Eicher, McAlester and Rottman is to be welcomed because, within its limitations, it copes very well with a difficult task. The principal limitation is one of brevity; the book forms part of a Prentice-Hall Earth Science series which is restricted to short, small-format books. The text is clearly written in plain, straightforward English and there are plenty of good illustrations. After an initial outline of stratigraphic principles and geochronology the authors deal with the Earth as a planet in a way more customary in physical geology texts, before devoting successive chapters to the Precambrian, Palaeozoic, Mesozoic and Cenozoic.

The writing is thoroughly up to date, whether dealing with subjects as different as Precambrian life and the cause of mass

extinctions, suspect terrains or orbital cycles as a cause of ice ages. The principal shortcoming is that the book is written at a very elementary level and has a minimal bibliography. There is little critical evaluation or in-depth treatment of specific subjects, and detailed examples are taken almost exclusively from the United States. This is essentially an Earth history digest aimed at the large American freshman and sophomore market; in consequence, it is unlikely to be adopted widely for geology honours courses in British universities, where world history is normally dealt with at a comparatively advanced level after a thorough grounding in stratigraphic principles has been undertaken with reference to local examples.

For the past eight years the most suitable textbook for senior undergraduates has been Windley's *The Evolving Continents*, in which a pioneering attempt was made at an overview of continental evolution from the Archaean onward, within a conceptual framework of plate tectonics. Windley's book has been a well warranted success and the second edition is timely.

So rapid have been the advances of recent years that substantial revision has proved necessary, though the basic outline remains unchanged. The new Harland timescale is adopted uncritically in a new opening chapter. It is perhaps as well that Windley did not wait a further couple of years before revision, because he would then have had three other new timescales to contend with, all differing in some important respects. Besides major changes in the Precambrian chapters there are new sections for the Phanerozoic on a host of subjects. These include, among many others, phosphorites in relation to oceanic upwelling, mass extinctions, the Iapetus Ocean, the elevation and climate of Pangaea, oceanic mineralization, Pacific island arcs, suspect terrains and the structure and evolution of the Himalayas. The bibliography remains at 30 pages but the contents are much changed.

As in the first edition, Windley is at his most authoritative when dealing with tectonics, especially the Archaean and Proterozoic, though he is not sufficiently up to date to record that much of eastern Asia is now thought to consist of isolated continental fragments brought together since the late Palaeozoic. On subjects such as palaeoenvironments, or those dealing with events in the biosphere, he is less comprehensive and reliable. The big advances recently in the understanding of sedimentary basin formation and continental margin subsidence are largely ignored, and Phanerozoic sea-level changes receive only a scant treatment. It would, however, need a superman to produce an advanced textbook of Earth history that was equally strong in all fields. □

A. Hallam is Lapworth Professor of Geology at the University of Birmingham.

Student pollution

Peter S. Liss

Fundamentals of Air Pollution, 2nd Edn.
By Arthur C. Stern *et al.*
Academic: 1984. Pp.530. $39.50, £28.
Pollution of our Atmosphere.
By B. Henderson-Sellers.
Adam Hilger: 1984. Pp.210. Hbk £30, $49; pbk £13.95, $23.

AIR POLLUTION is a fast-moving topic which has attracted much public attention in recent years. Developments in it have often been haphazard since, instead of a steady flow of funding for research, there has been a tendency to throw large amounts of money at particular problems, for example acid rain, and halocarbons and their possible impact on stratospheric ozone. With this sort of patchy temporal progress it might be thought that there would be widespread disagreement as to what should be included in the corpus of knowledge constituting the subject. However, to judge from these two books there is apparently considerable unanimity as to what courses in air pollution in higher education should contain. Basically both of them deal with sources, effects, measurement, monitoring, meteorology, and regulatory and engineering control of air pollution. But there the similarity ends.

The book by Stern *et al.* is a new edition of a publication of the same title which appeared 11 years ago. Two of the original four authors remain and two new ones have been recruited. The style and level of the new edition are much as previously, but the text has not merely been revised and considerable parts of it are completely new. Even so, there is a less than modern air about it, a possible reason for which will be discussed shortly. Students will find the book packed with information and it will serve them not only while they are at college but also in any subsequent career concerned with atmospheric science.

Henderson-Sellers's book is definitely a new production but again it doesn't strike me as really being at the forefront of knowledge. It is readable and a real attempt is made to explain how physical processes operate in the atmosphere, rather than merely stating that they do, as is done in less didactic treatments. The book is quite short and students should find it a good read, though I'm not sure it will be as useful to them after graduation as will Stern *et al.*

My principal grouse about both books is their lack of treatment of atmospheric chemistry. Both attempt to deal with chemical aspects of the subject but the approach is peripheral rather than central. Of the two, Stern *et al.* does a better job in this regard but even here the chemistry has insufficient weight. Because of this, important topics such as removal of air pollutants by natural cleansing processes, hydroxyl chemistry, and even acid rain receive only scant attention. It is the underplaying of chemical aspects which I believe gives both books a somewhat dated air. Arguably the biggest growth area in air pollution research in the past decade has been in atmospheric chemistry. Books in which insufficient attention is paid to this will inevitably look old before their time. □

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Archaeology anew

Colin Renfrew

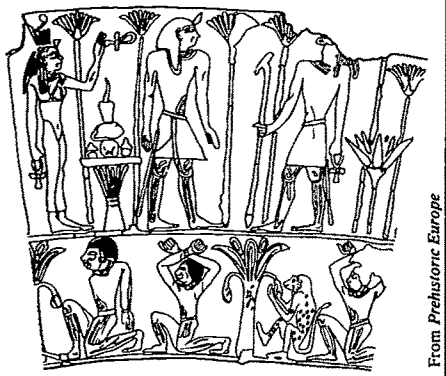
Prehistoric Europe.
By Timothy Champion, Clive Gamble, Stephen Shennan and Alasdair Whittle.
Academic: 1984. Pp.359. Hbk $45, £28.50; pbk $23.50, £14.50.

WHEN a discipline has recently undergone a profound, perhaps revolutionary transformation, the first textbook to follow that upheaval is of more than usual significance. The study of European prehistory has indeed undergone such a transformation over the past 15 years, with the widespread acceptance of a chronological system very different from that which prevailed earlier, and with the development of interests and forms of explanation which were not much in evidence then. This ambitious volume offers the first reasonably comprehensive overall coverage of the resulting subject, from the palaeolithic period to the iron age.

Three qualities separate the prehistoric archaeology of today from the traditional version, seen in a series of standard works from V. Gordon Childe's *Dawn of European Civilisation* (first edition 1925) to Stuart Piggott's *Ancient Europe* (1965). The first is the happy lack of preoccupation with chronology: radiocarbon dating has now successfully solved many of the outstanding chronological problems. So that while the early works of the new radiocarbon era were backed up by bulky appendices of date lists, much needed at the time, these are absent here. A second feature is the refreshing absence of automatic diffusionist assumptions: no longer is it taken as self-evident (whether by explicit axiom or by unstated convention) that most changes in the prehistoric record came about as the result of contacts with the civilized lands of the Near East by a process of the "diffusion" of culture. And thirdly, the processual approach now predominates: that is to say the emphasis is on the explanation of change through an analysis of economic conditions and social structures. The discussions of change in

this volume are lively and interesting, and in some cases new and original as well.

The book is the collective work of four authors: justifiably so, for there is no one today who could cover the subject single-handed with the same authority. Indeed perhaps there never was, for the earlier textbooks in general did not venture very far back into the palaeolithic period. Inevitably, however, in the necessary work of compression some detail is lost. We no longer see the comprehensive summaries of pottery styles and of the artefact forms



From *Prehistoric Europe*

Pictures of the past — decoration on a vase dating from the late eighth or seventh century BC found at Tarquinii in Etruria.

which occupied so large a proportion of many earlier accounts. Instead the themes treated are sometimes at a further remove from the raw data. In consequence this book will perhaps be more vulnerable to change as problems come to be solved and new questions arise. But, in a sense, the better the textbook the more rapidly will it be affected by the progress of current research.

The book is well-organized chronologically, and the treatment wisely takes the survey down to the beginning of the modern era (the BC - AD interface) for Europe as a whole. The emergence of the civilization of classical Greece thus falls within its scope, together with that of the Etruscans of Italy. This presents problems of treatment, which many earlier authors have avoided, since of course the availability of written sources brings Greece out of prehistory and into the light of history at that time, and the summary offered here will no doubt find its critics. But the solution is a bold one, and I find it altogether effective.

Minor faults and failings in several places can be rectified in a second edition. Meanwhile it is a pleasure to salute the appearance of a survey which for the first time since the New Archaeology of the 1960s presents the prehistory of Europe in a coherent and satisfying way. It will at once take its place as a new standard work, for it is quite simply the best introduction to European prehistory which is currently available. □

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Fossil selection

W.S. McKerrow

Atlas of Invertebrate Macrofossils.
Edited by John W. Murray.
Longman/Halsted: 1985. Pp.241. Pbk £13.95, $24.95.

IT IS very seldom that I have opened a new book with such delight. About half of its pages consist of magnificent photographs of well-preserved fossils, and primary congratulations must go to the photographers who have so ably produced these illustrations. The preface informs us that there are about 3,000 named minerals, but that the invertebrate macrofossils include tens of thousands of genera and hundreds of thousands of species. It has never been easy to select what to teach from this myriad. Now we have a choice of 900 well illustrated fossil genera, more than enough for an undergraduate course. Some of my favourite genera are missing, but most are there; more significantly only one or two minor groups of macrofossils are absent: hyolithids and archaeocyathids seem to have escaped the editor's net. (Vertebrates and microfossils are also absent, but by deliberate decision. Do they warrant two more books along the same lines?)

Seven of the contributors are from British universities, five from the Natural History Museum, one from the Geological Survey of Greenland and one from Brigham Young University, Utah. They all know what is needed to give authoritative descriptions of the common fossil groups. Although there is some bias towards north-west Europe, the fossils illustrated and described include genera from all over the world. So the book will be a useful text for any student in any country who can read English, while the diagrams and photographs should even prove helpful to those with only a scanty knowledge of the language.

Most chapters include a glossary of descriptive terms, and also an evolutionary diagram showing the relationships of the larger taxonomic groups. But, as the editor points out (p.2), "taxonomy is not the main aim of palaeontology. It is the application of correctly identified fossils to the problems of biostratigraphy and palaeoecology which makes palaeontology a major part of geology". The teacher who uses this book in class will still have plenty of work to do, especially in explaining the biology, ecology and evolution of these extinct animals. However, this atlas will make his task (and that of the student) so much more pleasant and so much more efficient. □

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A view of solids

R. Rudham

Solid State Chemistry and its Applications.
By Anthony R. West.
Wiley: 1984. Pp.734. £37, $67.95.

HAVING carefully defined solid state chemistry as the synthesis, structure, properties and applications of solid materials, the author of this book is somewhat confined within a straitjacket of his own manufacture. Unfortunately, his definition precludes consideration of the important interactions between solids and gases or liquids. The fact that the book has nothing to say about adsorption and heterogeneous catalysis is perfectly acceptable, but when metal oxidation is similarly ignored one cannot help questioning the desirability of such a definition of solid state chemistry.

The non-mathematical approach adopted by the author contributes to the readability of his work, but inevitably introduces some shortcomings. For example, the law of mass action treatment given for lattice defect equilibria is less informative than that of statistical thermodynamics, where the compensating contributions to the overall free energy from defect formation and configurational entropy are directly seen. Nevertheless, the chapters on structure and properties present an interesting and coherent account of these fundamental aspects.

What is less certain is the merit of the last four chapters on glasses, cements, refractories and organic solids, when serious consideration of diffusion processes and solid state chemical reactions has been omitted. In particular, the final chapter on the organic solid state seems something of an afterthought and in its brevity unjustly emphasizes the inorganic nature of the subject. To be fair, the early chapter on synthetic methods presents information on chemical reactions involving solids, but also injudiciously absolves the book from consideration of their kinetics. It is in this chapter that one of the major founders of modern solid state chemistry, Carl Wagner, receives his only mention.

West has written a remarkably readable account of solid state chemistry as he sees it, but who will read it? Both size and price will preclude its formal adoption as an undergraduate text, although it should make the recommended reading lists. Postgraduate students and other researchers will find it gently informative on areas other than those of their personal endeavours. The most probable readership will be non-chemists who have reason to enquire into the nature and physical behaviour of solids at the atomic level. □

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Different elements to chemistry

Jon McCleverty

Inorganic Chemistry: Principles of Structure and Reactivity, 3rd Edn.
By James E. Huheey.
Harper & Row: 1984. Pp.936. Hbk $37.50; pbk £11.50.
Chemistry of the Elements.
By N.N. Greenwood and A. Earnshaw.
Pergamon: 1984. Pp.1,542. Hbk £75, $120; pbk £19.50, $34.95.
Structural Inorganic Chemistry, 5th Edn.
By A.F. Wells.
Clarendon: 1984. Pp.1,382. £75, $98.

OF THE three books reviewed here, two are, to most inorganic chemists, old friends (Huheey and Wells), and one is a keenly anticipated newcomer. The books by Huheey (now in its third edition) and Greenwood and Earnshaw are intended as student texts, whilst that by Wells (a fifth edition) is primarily a work of scholarship and reference directed at one aspect of inorganic chemistry — the structure of crystals and solids of all types.

I have always been an admirer of Huheey's account of the principles of structure and reactivity in inorganic chemistry, largely because, like my students, I found it immensely readable and a nicely presented package of material for the core of the first two years of an undergraduate course. Typically North American in its approach, combining a distinct flavour of the physical chemist's view of chemistry, nonetheless it advances inorganic chemistry within a topic approach which makes the consumption of a wide range of facts both appetizing and digestible.

The early chapters deal with atomic structure, bonding models, chemical forces, acid–base chemistry and chemical behaviour in aqueous and non-aqueous media. It is gratifying to see a chapter on solid-state chemistry, although perhaps rather small in the light of the rapid growth of interest in the subject. Later chapters are concerned with coordination chemistry in all its aspects, organo-transition metal chemistry (including ritual obeisance to homogeneous catalysis) and bioinorganic chemistry. There are three chapters covering some descriptive chemistry of the d- and f-block metals, and of the halogens and rare gases. This is an improvement over the earlier editions, although there are, inevitably, still quite significant gaps. To some extent, however, the omissions are partly catered for in the excellent chapter on periodicity. The appendices contain a wealth of physical data, and discussions of nomenclature and of group theoretical symbolism and symmetry (although this is not used specifically in the text).

Greenwood and Earnshaw provide a wholly different menu of inorganic chemistry, returning in a sense to a factual presentation based on single elements or groups of elements following the periodic table. Thus the s- and p-block elements take up most of the first half of the book, and transition metals, reported by vertical groups, the second half. The only time that topics are discussed is at the beginning — the origin of the elements, isotopes and atomic weights, and chemical periodicity and the periodic table — and later with an account of coordination chemistry which precedes discussion of the transition metals.

Within each chapter, the properties of the elements and their compounds are discussed systematically, and aspects of solid state, organometallic and bioinorganic chemistry are referred to at the appropriate point. An unusual and unexpected feature is the inclusion in the chapter on carbon of a major discussion of metal carbonyl and hydrocarbon chemistry, particularly from the structure and bonding point of view. But by far the most interesting and novel feature of this book is the liberal presentation of facts and figures on the uses and potentially useful properties of inorganic compounds, information which is almost totally absent from any other major texts in this subject area. The danger of including such material is, of course, that it may rapidly become out of date, but that problem apart this is a fresh approach to the furthering of the relevance of inorganic chemistry which is to be welcomed enthusiastically.

It is not helpful to say which of the two books one might choose as the basis for a course of inorganic chemistry at the tertiary level. Huheey is certainly appropriate for the first two years of a British undergraduate curriculum, and I suspect that students would prefer it initially as a more helpful study guide. However, Greenwood and Earnshaw's book unquestionably contains more chemical information, and course instructors will be immensely grateful to the Leeds chemists for having reminded us all that facts are facts, whereas theories often reflect passing fashions and fancies.

Wells's *Structural Inorganic Chemistry* cannot be realistically regarded as a student text, although senior undergraduates will undoubtedly use it for reference. It seeks to present the structural basis of inorganic chemistry for chemists, and it succeeds. It is a little bit like an Irish stew — everything is included, cooked in a special and uniform way, making up a rather fine and nourishing dish.

The book is divided into two parts, the first of which deals with a number of general topics, including the properties of polyhedra, the nature of symmetry and repeating patterns, the way in which spheres of varying sizes pack together, and bonding in molecules and crystals. The second, longer part describes the structural chemistry of the elements, based on the groups of the periodic table. Here oxides, hydroxides, aquo species, sulphides and halides receive considerable attention, but there are also chapters dealing with metal carbonyls, carbides and alkyls, the coordination chemistry of the later transition metals, and metals and alloys. The text is usefully referenced and there are formula and subject indexes.

In a subject as vast as structural inorganic chemistry, encompassed in some 1,400 pages of text, there are bound to be some topics which receive only cursory treatment. However, this is likely to worry only the crystallographer rather than the broader congregation of chemists, and the author is to be congratulated on assembling such an amount of useful information in such an accessible form. □

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Organic affairs

John Mann

Advanced Organic Chemistry, Part A, 2nd Edn: Structure and Mechanisms.
By Francis A. Carey and Richard J. Sundberg.
Plenum: 1984. Pp.726. Hbk $59.50, £56.53; pbk $16.95, £16.19.
Organic Chemistry.
By G. Marc Loudon.
Addison-Wesley: 1984. Pp.1,451. $39.95, £39.
The Third Dimension in Organic Chemistry.
By Alan Bassindale.
Wiley: 1984. Pp.242. Pbk £7.95, $17.95.
Spectral Problems in Organic Chemistry.
By R. Davis and C.H.J. Wells.
Chapman & Hall/Methuen: 1984. Pp.121. Pbk £5, $10.95.

LIKE Shakespeare's *Henry IV*, *Advanced Organic Chemistry* by Carey and Sundberg comes in two parts. Last year (*Nature* **308**, 136; 1984) I extolled the virtues of the second edition of Part B, which is concerned with the methodology of organic synthesis. Part A deals with structure and mechanisms and, like its companion volume, is aimed at the advanced undergraduate or novice graduate student.

The style is modern, and both molecular orbital theory and the frontier orbital approach are used to good effect. There is a lot of physical organic information pertaining to the various reaction types, and other highlights include chapters on stereochemical principles and conformational analysis, concerted reactions and photochemistry. The numerous problems provided are well-chosen, and many contain data taken from the research literature.

Overall, the book complements Part B

most satisfactorily — the one providing the theory, and the other the practice. There is, however, a rival text of the same title by Jerry March, published by McGraw-Hill, a third edition of which is due later this year. March has become an essential purchase for many graduate students, especially for its vast collection of literature references. Carey and Sundberg's books are more readable and much better illustrated, but are not so well endowed with references. Any decision between the two types of book will depend upon personal preference and the size of one's bank balance (assuming that one book costs less than two).

At a lower level there are now so many books on organic chemistry that any new contender must offer something different in order to succeed. Recent innovations include two-tone illustrations, comprehensive chapters on spectroscopic methods and sections on "special topics". In his book, Loudon incorporates the first two of these, but spurns the last, somewhat gimmicky one.

Most books of this kind commence with the alkanes and introduce mechanistic concepts through a consideration of free radical processes. In contrast, Loudon believes that the "curly arrow" formalism and heterolytic processes should be used as an introduction to mechanistic organic chemistry, and I suspect that many would support this. Two-tone illustrations are used to good effect, not only for mechanisms but also whenever emphasis is required, as, for example, in the chapter on NMR spectroscopy. This appears early in the book, and stresses the practical uses (with lots of examples) rather than the theory. The links with life processes are not forgotten, so we find biological oxidation alongside oxidation of alcohols; ionophore antibiotics in with the ethers; and the mode of action of S-adenosyl methionine as an example of sulphonium chemistry. In fact the chapter on organosulphur chemistry is more extensive than in any other recent text of this kind, and reflects modern usage of these reagents. The same can be said of the account of heteroaromatic species. Other nice touches include examples of experiments, amusing historical anecdotes and end-of-chapter summaries.

There are, however, some important omissions — for example pericyclic reactions and organophosphorus chemistry — and there is a rather limited treatment of the strategy of organic synthesis. In these respects Streitwieser and Heathcock's *Introduction to Organic Chemistry* (Collier Macmillan/Macmillan 1981) is superior. Omissions apart, Loudon's book is beautifully presented, has lots of interesting problems and is very readable.

The Third Dimension in Organic Chemistry by Alan Bassindale is not an easy read, but the effort is well rewarded. It is much more than a text on the shapes of carbon compounds, and covers all of stereochemistry including the dynamic aspects. The book is divided into three almost equal parts, and commences with a section on general stereochemical principles which takes the reader from a pre-university level as far as elementary conformational analysis. Part 2 deals with conformations of acyclic and cyclic systems and introduces the concept of chirality. This prepares the reader for the meatiest part of the book which covers the stereochemical features of the main reaction types. Substitution, addition and elimination reactions are discussed in detail, and there is also a chapter on pericyclic reactions. Finally, the concept of asymmetric synthesis is introduced and this is most pertinent since so much modern synthesis is concerned with the quest for stereochemically pure products. This chapter should perhaps have been longer, however, with a few more practical examples, such as the use of metal enolates or chiral hydrazones in asymmetric synthesis. One other suggestion for the second edition would be the provision of a decent stencil for the chair conformations depicted! Otherwise, the book is an excellent introduction to stereochemistry and should become very popular.

This should also be true of the book by Davis and Wells, which provides 56 spectral problems at a cost of about 9p a time. Infrared, NMR (proton and ^{13}C) and mass spectral data are given for each compound, and in most cases an elemental analysis is also supplied. The answers are given in the form of references to *Beilstein*, the *Merck Index*, the *Dictionary of Organic Compounds* or to Aldrich's chemical catalogue. The spectra are reproduced well and the problems are both interesting and demanding; this book is especially welcome because interpretation of spectra (in particular NMR spectra) is nowadays central to most courses in organic chemistry. □

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Textbook supplement — prices

Where possible both dollar prices in the United States and sterling prices in Britain are given in the bibliographical details for each book. Export prices will generally be higher. If a dollar or sterling price is not cited, the book is not available in one of the two countries. However readers should check both price and availability of books before ordering.

Metabolic change

M.L. Sinnott

Essentials of Bio-organic Chemistry.
By R.W. Hanson.
Edward Arnold: 1984. Pp.208. Pbk £6.95, $14.95.

OVER the past four or five decades, the application of chemical techniques and concepts to biology has proved one of the most fertile fields of scientific endeavour. Undergraduates in the biological sciences should now, ideally, have a firm grounding in those areas of chemistry germane to their studies: thus biochemists should understand the chemical logic behind metabolic transformations. Dr Hanson has taken "bioorganic chemistry" to mean the material necessary for an understanding of the chemistry of the transformations of organic metabolites *in vivo*.

The ground the book attempts to cover is very well chosen — a treatment of the properties of biologically occurring functional groups, uncluttered by the recondite technology of Synthesis, is followed by chapters on enzymes and coenzymes. However, in execution the text is a catastrophic failure with serious errors of fact and comprehension throughout.

For example, we read that "in general all positively charged electrophiles are more powerfully electrophilic than neutral compounds" (p.24); that ΔG^* ($\Delta G^{\ddagger}$?) is "a measure of the energy which must be associated with a collision between A and B" (for reaction to occur) (p.28); that "oxidations are invariably associated with a large negative value of ΔG" (p.36); that, in the context of *aliphatic* diazonium ions, aromatic diazonium ions undergo nucleophilic displacements "for example, with iodide ions" (p.75); that "the 'entropic effect' of enzymes may cause rate accelerations of 10^8" (not 10^8M); and that "electronic catalysis might contribute a further factor of the order of 10^2" (p.154). Dr Hanson's treatment of additions to the carbonyl group (p.87 ff.) is innocent of any mention of general acid–base catalysis, his discussion of prochirality does not distinguish between enantiotopic and diastereotopic groups, and the concept of transition state analogues materializes without explanation from "application of transition state theory to . . . enzymes" (p. 164).

Although wholly inadequate in matters of substance, the text pays much attention to nomenclature and IUPAC names are used for small molecules (propanone for acetone, 2-amino acids for α-amino acids): of course this scientific Volapük is impracticable for large biological molecules, which are still called by the names people use. So we see "methanoic acid" on p.103 but "formyl methionine" on p.107.

The standard of scholarship of this text holds a moral for those of our political masters who wish to grind down more of the tertiary education system to a "teaching only" function. Even if an academic has the insight to identify a real educational need — as Dr Hanson has done — that need is most unlikely to be filled unless the academic has a substantial research presence in the area concerned. □

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Animals illustrated

D.R. Newth

Integrated Principles of Zoology, 7th Edn.
By Cleveland P. Hickman Jr, Larry S. Roberts and Frances M. Hickman.
Times Mirror/Mosby/Blackwell Scientific: 1984. Pp. 1,065. $34.95, £38.
General Zoology, 6th Edn.
By Claude A. Villee, Warren F. Walker Jr and Robert D. Barnes.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.992. Hbk £22.95, $36.95; pbk £18.95.

EACH of these texts is about thirty years and half-a-dozen editions removed from its first appearance. Each is the work of three authors who acknowledge help in revision from many sources. Each is large and lavishly illustrated, devotes its largest single section to a systematically arranged account of the animal kingdom, but gives over half its total length to sections on behaviour, cell biology, ecology, genetics and physiology. Both sets of authors, in fact, have tried to include in one volume the minimum background of chemical and biological science necessary to make sense of any, or all, forms of animal life. In consequence they have had to be sparing in their descriptions of actual animals. The balance struck in both books is, I think, about right for texts at this level.

It would be surprising if many of the idiosyncracies, personal opinions on matters in dispute, or even the occasional lapses in matters of fact or of taste which endear the single-author book to the reader, could survive the editorial process in a middle-aged text produced for large numbers of undergraduate students. These losses are the price we pay for a gain in reliability and for a reduced danger of major omission. These two books, having set out with similar aims, have adopted broadly similar methods and have succeeded to much the same extent.

However the two are not indistinguishable. Hickman *et al.* is the more relaxed in tone, the more generously produced and, perhaps, the more willing to comment, from the zoologist's point of view, upon matters of public or lay interest. Thus the book ends with a chapter on animals in the human environment which gives a balanced but forceful treatment of the need to adopt conservation measures worldwide and without delay. Old-fashioned textbooks might mourn the extinction of species, but would hesitate to quote specific legislation and litigation in describing any one case. The story of the attempts to save the snail darter from the effects of building the Tellico dam may well serve to excite more interest in that unfortunate fish than it would ever otherwise have received.

Hickman *et al.* is the more colourful book in the literal sense, using colour photographs freely and sometimes to pleasing effect. Whether or not this always adds to the value of the illustration is perhaps doubtful. It also evades the modern publisher's unhappy proscription of footnotes by allowing use of the wide margins of the printed page for "marginal notes" in which additional or peripheral matter can be added without stemming the flow of the main story. A similar, but less sightly, trick is used by Villee *et al.* who place such matter in "boxes" which can take up much of the page. This apart Villee *et al.* is altogether more reserved in presentation, both visually and in the text. It provides a businesslike account of its material, but must be faulted for a rather cavalier treatment of some important areas. Parasitology, the insects of economic importance, indeed the insects as a whole, and regeneration are cases in point. While my own preference would be for the more lively and imaginative approach of Hickman *et al.*, there is not a great deal in it. □

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Aspects of insects

M.E.G. Evans

Insect Biology: A Textbook of Entomology.
By Howard E. Evans.
Addison-Wesley: 1984. Pp.436. $32.95, £14.95.
Insect Physiology, 8th Edn.
By V.B. Wigglesworth.
Chapman & Hall/Methuen: 1984. Pp.191. Hbk £15, $35; pbk £7.95, $15.95.

INSECTS are now studied as living animals rather than as stamps in collections, and this changed attitude is reaching student texts. Both of these books deal with insects as living organisms: their forms, functions and lifestyles. The first book is a fairly large general text, which concentrates particularly upon the behaviour, physiology and ecology of insects, whilst the second is a concise introduction to their physiology.

Some years ago, H.E. Evans wrote what was probably the best recent book on insects which was comprehensible to the general public (*Life on a Little-known Planet*, published by Dutton in 1968), so I received his textbook with great interest and anticipation. In fact, this is a multi-author work, as Evans has five colleagues as co-authors, but the style is consistent and a high standard of clarity is maintained throughout.

The 18 chapters are grouped into seven parts dealing respectively with structure and diversity, functions and development, behaviour, plant–insect relationships, relationships with animals, ecology and pest management. There is also an appendix on non-insectan Arthropoda which is followed by a useful glossary. Each of the chapters concludes with a summary followed by "selected readings"; these include the most pertinent references, but might have been longer and more comprehensive. With few lapses, the text is written simply and informatively, and the illustrations are mostly straightforward line diagrams which are neatly boxed to separate them from the text. The traditional entomological preoccupations with morphology, taxonomy and diversity have been condensed into the first section to allow for expansion of the later parts.

Wigglesworth's book is a new edition of a well-established work; indeed, there can be very few authors who have produced a new edition of their book a full half-century after the original date of publication. It covers the traditional range of insect physiology by describing the operation of the various organ systems (many of which have been elucidated by Sir Vincent himself over considerably more than the past 50 years). Although similar to earlier editions, much new material has been incorporated, and more subheadings have increased the accessibility of different subjects in the text. The references have been updated and many new figures added, while the narrative is still admirably readable and comprehensible.

Insect Physiology was originally a pioneer in the Methuen *Monograph* series — little books which were a great boon to earlier generations of student biologists who lacked the huge diversity of modern textbooks. But even if the texts reviewed here are both good in their different ways, do modern students need yet more books, recycled or new? If an established text can be revised adequately, there is good reason to keep it. Wigglesworth's small (but expanding) book probably is most useful in providing an introduction to insect physiology for non-entomological zoologists, or to whet the appetite of entomology students at an early stage of their training. More advanced students will need his *Principles of Insect Physiology* (Chapman & Hall, 7th Edn 1972), or an equivalent expanded treatment.

A new entomology text must provide a fresh look at the subject in order to compete, and Evans has had the advantage of being able to take a contemporary viewpoint throughout. *Insect Biology* would provide an excellent basis for a modern entomology course taught as one of several subjects for a zoology or biology first degree. For a more specialist entomology course, morphological and systematic reinforcement would be necessary, as provided, for instance, by Borror, DeLong and Triplehorn's *An Introduction to the Study of Insects* (Holt, Rinehart & Winston, 5th Edn 1981) or *Imms' General Textbook of Entomology* (Wiley/Chapman & Hall, 10th Edn 1977).

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Introducing biology

E.C. Cox & R.M. May

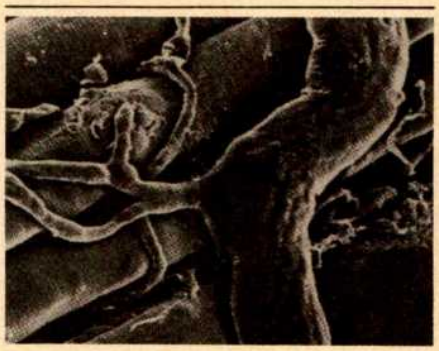
Exploring Biology, 2nd Edn.
By Pamela S. Camp and Karen Arms.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.591. £27.95, $32.95.
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By Thomas A. Easton and Carl E. Rischer.
Charles E. Merrill: 1984. Pp.719. Hbk £16.95; pbk $19.95.
Biology: The Unity and Diversity of Life, 3rd Edn.
By Cecie Starr and Ralph Taggart.
Wadsworth: 1984. Pp.697. $29.10, £49.60.
Biology: An Introduction.
By Kenneth D. Johnson, David L. Rayle and Hale L. Wedberg.
Benjamin/Cummings: 1984. Pp.615. $28.95, £25.15.

ALL four of these texts are intended for students who will probably take only one biology course and whose teachers are interested in covering the broad range of topics now typically found in standard texts for science majors. Three of them follow what many have come to accept as the preordained little-to-big (molecules to ecology) order of presentation. The exception is Camp and Arms (CA), whose book begins with evolution and ecology, and then proceeds to cells, genetics, plants and animals; this book is essentially a scaled-down version of the same authors' *Biology*, a well-written text for science majors.

With the exception of Easton and Rischer (ER), which has an unfortunate number of errors, these books are scholarly, accurate, up-to-date and well produced — Starr and Taggart (ST) exceptionally so —, including thorough coverage of plant biology, and good discussions of human anatomy and physiology. Although intended for a one-term survey course, they treat the readers seriously, something we found lacking in similar texts when we reviewed this field several years ago. The books have a seriousness of purpose and intellectual rigour that is easily lost when authors try too hard to enliven their subject by making it trendy and "relevant" (and even here there remain lapses from grace, such as the photo in Johnson, Rayle and Wedberg (JRW) captioned "the rock star Johnny Winter is an albino").

ST is an extraordinarily well illustrated book, with figures and photographs that rival those in Curtis's *Biology* for clarity and sheer beauty. The other three books are illustrated well, but none comes close to ST. All the books end each chapter with review questions and a list of suggestions for further reading. These lists are a bit dated, idiosyncratic and exhibit the usual high coefficient of inbreeding with other introductory texts.

The evolution and ecology sections do not strike us as the strongest parts of any of the four books. In this respect, none of them is up to the standards set by Purves and Orians in *Life: The Science of Biology*. There are, however, interesting differences among the four. JRW and ER illustrate speciation with the rather tired example of the beaks of Galapagos finches; CA uses the more compelling example of the Hawaiian honeycreepers; ST also uses the Hawaiian honeycreepers, but with an illustration that is more complete and more beautiful than that of CA. All four books discuss continental drift and its relation to past and present biogeographical patterns, but while the other three show the usual picture of continental drift over the past 200 million years, ST have a new double-page spread correlating the evolution of life with the location of the continents since the late Cambrian (540 million years ago). On the other hand, ST is the only one of the books to perpetuate the appealing but incorrect generalization that roughly 10% of the energy at one trophic level is transferred to the next (it is one of our pet peeves that this "law" is so long in dying). On balance, however, ST seems to have made the greatest efforts to break out from the incestuous patterns — the convergence in use and reuse of familiar examples — that afflict most introductory texts.



Nerve fibres on muscle (x900). The picture is taken from Biology *by Claude Villee* et al., *published by Holt Saunders/Holt, Rinehart & Winston.*

All the books make efforts to incorporate recent advances, going beyond the molecular biology of the gene to the molecular biology of the cell. But the text fully bringing an abstract of Alberts *et al.*'s *Molecular Biology of the Cell* into the introductory classroom remains to be written.

More generally, it seems that if an elementary text is to give some idea of the great diversity of methodology and levels of analysis currently found in the biological sciences, it must sacrifice something in the telling. In the books reviewed here, what is lost is a feeling for the way in which science is done and for the elegance of many experiments. It seems to us that this is inevitable in a non-trivial book for a one-term course, where the emphasis is on scientific content. Whether or not this is how non-science majors should be taught has always been a matter for lively debate, and could well be the subject of a different essay.

None of these books spends more than a sentence or two on creationist theories. Only JRW includes a reference in the index, and the authors briefly and sensibly say that all such conjectures are matters of faith outside the realm of science. This represents a change in college texts over the past several years. Not long ago authors felt compelled to defend Darwinism against attacks from modern proponents of special creation (with Luria, Gould and Singer's *A View of Life* being a high-water mark). Happily, this is no longer so if these texts are at all representative. The poverty of special creation theories as scientific explanation is taken as given.

Broadly speaking, the intrusion of the social and moral concerns of textbook writers into purportedly objective texts must surely date from earliest times. In this respect the books under review also represent a change from recent crops, in that they mainly focus on science, tending to leave out issues that always have seemed to us more properly contained in social science texts. No doubt there will continue to be introductory texts on the market that grip the student with rhetorical pleas for the state of the environment or the brotherhood of man (or siblinghood of persons), but these are not among them. There are lapses, such as ER ending with the time-honoured two views of the future (replete with imaginative illustrations of how "the movement to space could begin with the construction of orbiting space colonies"), but in the main we have factual accounts that balance the many disciplinary calls on the student's attention with the need to remain comprehensive and easy to read.

All in all, we think ST comes out ahead. CA and JRW are both good, with JRW deliberately aiming a bit higher (for example, it gives the mathematics of the Hardy–Weinberg law, while CA does not). ER is pitched a bit lower than the other three, and contains quite a few serious errors and misleading figures: for instance, on page 115 the two polynucleotide chains of the double helix have the same polarity, while the codon table on page 117 is badly scrambled. We like ST best because it is so well illustrated, succinct and marvellously clear. It also manages to cover more ground and to do the best job of the three in the sections on energetics and chemistry, while retaining its comprehensiveness and avoiding excessive length and detail.

Personal preferences notwithstanding, all but ER are good value. This may in part be because the authors have worked closely with their reviewers, as acknowledged in each preface. The majority of the reviewers involved have wide experience teaching the students for whom the books are written. All of us profit from this collaboration. □

Edward C. Cox and Robert M. May are Professors in the Department of Biology at Princeton University.

Vertebrates as a whole

R. McNeill Alexander

Vertebrate Natural History.
By Mary F. Willson.
Holt Saunders/Holt Rinehart & Winston: 1984. Pp.621. £33.95, $37.95.

"NATURAL history" is a term that needs rehabilitation. We tend to use it only to refer to amateur field studies, and in the names of museums, but it is an excellent, succinct description for studies that deal with whole organisms rather than tissues or cells. In *Vertebrate Natural History* Dr Willson uses it very broadly, to refer to subject areas ranging from environmental physiology to sociobiology. She tells us that "for the modern study of natural history, it is inadequate to construct a catalog of cute little tricks employed by organisms".

The book has four parts, which are not well integrated. The first is intended to set the scene, and includes elementary introductions to evolution and allometry, a review of the vertebrates (which is little more than a catalogue of orders, without anatomical detail) and a description of the world's climates and vegetation. The second part is the physiological one, discussing senses, thermo- and osmoregulation, respiration, locomotion and migration, while the third (and shortest) deals with foraging and escape from predators. The final part covers such topics as home range and territory, courtship and mating, life history patterns and parental care.

In this book we are told about a great many aspects of the lives of a great many animals, but there is little cross-reference between one chapter and another and we are not given an integrated impression of the life of any species. The text is superficial, citing a great many investigations but providing little information about any one of them. Explanations are generally facile, without adequate reference to basic principles, and are sometimes misleading. The experimental evidence for statements is seldom given.

There are many fine drawings and photographs of animals mentioned in the text, and the chapters are headed by delightful decorative sketches, but few of the diagrams are original. In some compensation for the vagueness of the text each chapter includes a long list of generally well-chosen references — a remarkable number of the best papers of the 1960s and 1970s are listed, but only a few from the 1980s. □

R. McNeill Alexander is Professor of Zoology at the University of Leeds.

After Darwin

R.J. Berry

Evolution: A Modern Synthesis.
By W.H. Dowdeswell.
Heinemann Educational: 1984. Pp.176. Pbk £6.50.

BIOLOGY first made sense to me when my A-level course reached evolution. The seemingly endless tread-mill of form and function, ethology and ecology, taxonomy and biochemistry suddenly became part of a coherent whole. I was fortunate in my teachers. Many students coming up to university have only a slight or garbled understanding of evolutionary biology; as a result they tend to believe that methodology-driven disciplines such as molecular genetics or behavioural studies are the real keys to biology. Anything that counters this false form of reductionism is good and it is a pleasure to see the appearance of Professor Dowdeswell's latest book.

Thirty years ago, Dowdeswell wrote a book called the *Mechanism of Evolution* in the Heinemann Scholarship Series. This was obviously a success because it went through several editions and reprints. His new book is presumably intended as a replacement. It includes chapters on the origin of life and the palaeontological record, but otherwise has a similar structure to the earlier work. But the new is not simply a rehash of the old: punctuationalism is discussed, as are cladism and creationism (the last not entirely satisfactorily; but it is refreshing to find a fundamentalist correctly defined as one who adheres to the fundamentals of Christian faith), and odd snippets of information crop up which identify the book as written by someone who knows the subject rather than an intelligent hack who simply recycles existing texts. (For example, sickle cell haemoglobin homozygotes are conventially regarded as having zero fitness, but Dowdeswell describes a Saudi population where fetal haemoglobin persists into adult life and increases the fitness of sickle homozygotes apparently to unity; and the influence of plant density on the frequency of primrose homostyles is described, taking the standard story of their maintenance away from mere speculation about the effects of inbreeding.)

Evolution is a text for sixth formers and for those undergraduates arriving at university with only a tenuous grasp of the subject. It is admirably designed for that readership, as would be expected from a teacher of Dowdeswell's experience. My only grouse is that it may be too placid: will it excite current generations of A-level candidates? I don't know; I can only hope.

R.J. Berry is Professor of Genetics at University College London.

An idea of primates

Michael Simpson

Primate Behaviour and Social Ecology.
By Hilary O. Box.
Chapman & Hall/Methuen: 1984. Pp.283. £10.95, $22.95.

THOSE who enjoy working with and reading about primates for their intrinsic interest will welcome this book as a rich vein of information and reference. Here is an uncontroversial source to complement those primate behaviour courses which concentrate upon just a few strong ideas, illustrated with a handful of examples, described in depth. It is indeed useful to have a psychologist's synthesis of approaches to "intelligent" responses to changes within social and physical environments, evident in the sections "Responsiveness and Life Strategies", "Self-awareness", "Piagetian Techniques", "The Use of Tools" and "The Question of Culture". Other kinds of material, such as Packer's study of male transfer in baboons, are well summarized for teaching purposes.

However, two *caveats* should be made in reponse to the book's advertisement of itself as a text for students. First, strong theoretical lines are not pursued. It could be argued that in so far as the study of primate behaviour is in a "fact-gathering hypothesis-testing" phase, this book's unfocused Aristotelian approach is appropriate, and most of the current ideas available (including so-called sociobiological ones) cannot do justice to the complex data. But a particular viewpoint can also sharpen our focus on factual material, and there is already a harvest of particular facts whose quiddity cries out for theoretical support. Why, for example, should chimpanzees and bonnet macaques be almost the only multi-male primate groups in which males groom each other extensively? Why do the daughters of so many macaque families rank socially in inverse order of their ages? Theories answering such questions could have much to say about social competition and co-operation.

My second reservation is that students will not always find that closer reading clarifies difficult passages. For instance, gender ratio adjustments in Japanese macaques (p.120) and vervet monkeys (p.233) are mentioned, but the directions of such adjustments cannot be found in the text. Fig.6.9 lacks a code for its letter symbols which would let it show "the situation clearly" (p.241). In some passages (for example p.43) the author is worsted in "the intolerable wrestle with words and meanings". □

Michael Simpson is in the Medical Research Council's Unit on the Development and Integration of Behaviour at the University of Cambridge.

Telling tales

Geoffrey Hall

From Darwin to Behaviourism: Psychology and the Minds of Animals.
By Robert Boakes.
Cambridge University Press: 1984. Pp.279. Hbk £35, $69.50; pbk £15, $19.95.

DISCUSSING the implications of his theory, Darwin wrote in the *Origin of Species* that "psychology will be based on a new foundation, that of the necessary acquirement of each mental power and capacity by gradation. Light will be thrown on the origin of man and his history". But the facts of psychology were to prove troublesome to Darwinian theory as he himself acknowledged in the *Descent of Man*: "the high standard of our intellectual powers and moral disposition is the greatest difficulty which presents itself, after we have been driven to this conclusion on the origin of man".

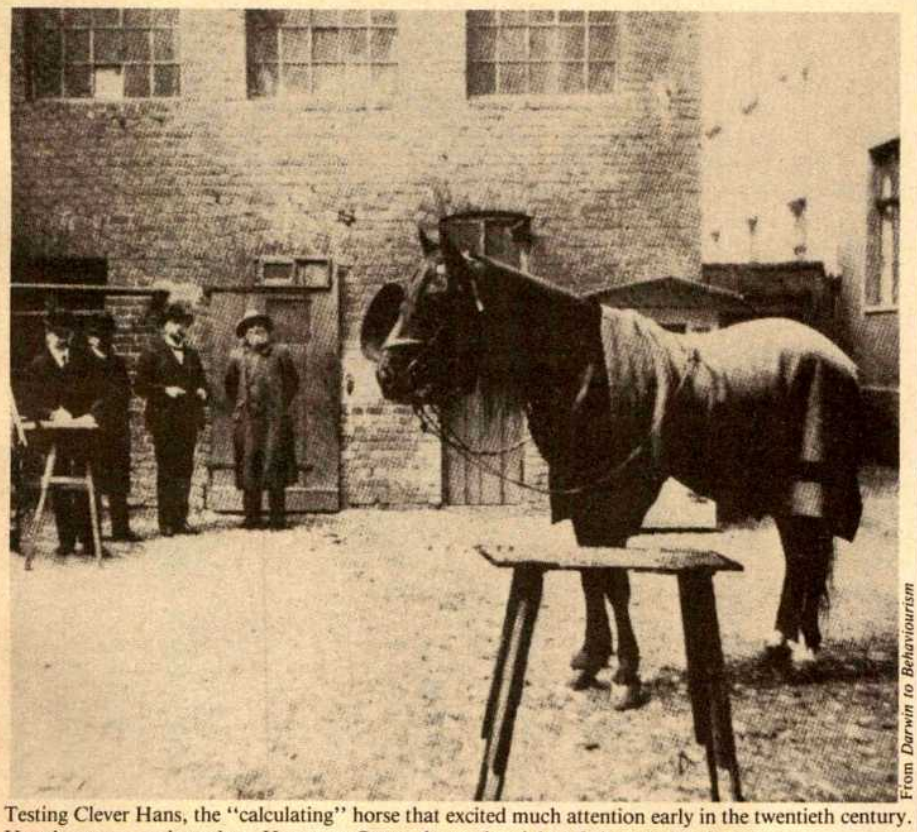
The problem for supporters of the "continuity hypothesis" was to find evidence for the existence of mental powers in non-human animals and this Darwin and his successors (notably G.J. Romanes and C.L. Morgan) set out to unearth. Their work forms one of the foundation stones of modern behavioural psychology and constitutes one of the major themes of Boakes' excellent book.

An earlier view of the nature of animal behaviour has its origins in Descartes' notion of them as reflex automata. The second theme of the book is found in Boakes' discussion of the concept of the reflex as the mechanism of animal behaviour from its Cartesian origins, through la Mettrie, Hartley and the work of nineteenth-century German physiologists, to that of Pavlov and associates.

The two themes come together in the final third of the book, when we move to the early twentieth century and the scene of intellectual activity switches, for the most part, from the Old World to the New. In the United States we see the creation of an explicit behaviourism, a view of psychology which, to put it crudely, accepted a reflex account of animal behaviour but which avoided any conflict with evolutionary theory by its willingness to apply (or try to apply) the same account to human behaviour. Boakes, of course, does not put it crudely and his thorough discussion of behaviourism and its implications should help to counteract the simplistic and condescending descriptions to be found in so many textbooks.

Altogether this book is a fine achievement. There is no doubting the depth of scholarship but this is also a good story, well told, which should appeal to psychologists and philosophers not primarily interested in the history of their subjects. And everyone will obtain pleasure and instruction from looking at the illustrations, many of them from private sources and quite new to me, the discovery of which must have been a formidable undertaking in itself. □

Geoffrey Hall is Senior Lecturer in Psychology at the University of York.



From *Darwin to Behaviourism*

Testing Clever Hans, the "calculating" horse that excited much attention early in the twentieth century. Hans's owner and teacher, Herr von Osten, is on the right of the group of men.

Psychometrics at the trot

Steve Blinkhorn

Psychological Testing.
By John R. Graham and Roy S. Lilly.
Prentice-Hall: 1984. Pp.433. $39.10, £35.60.

ON FIRST acquaintance with psychological testing there is a constant demand from students for emphasis on details of published tests and their application. On the other hand lecturers may very properly feel that a sound grasp of psychometric methodology is of more value in getting to grips with the often difficult and tendentious issues associated with the use of tests.

Graham and Lilly have resolved this tension in favour of the demand from below. After a quick, elegant canter round the basic statistical and mathematical concepts, the bulk of this book is given over to description and evaluation of published tests. The student is introduced to a range of issues via a discussion of particular tests, and appropriate primary sources are referenced. Concluding chapters present an overview of assessment as a process, and of controversial issues in the use of tests.

Within its self-imposed limits the book is admirably written, providing a smooth, well thought out account of most of the topics one would wish to include in an introductory course. This is a fundamental failing. There is no challenge left when every dilemma is polled, every contentious issue sanitized and a range of sound, sane opinion presented neatly gift-wrapped as if ready for instant regurgitation in a multiple-choice quiz.

This is not merely reviewer's dyspepsia: textbooks of this kind, for all their merits as digests, encourage amongst students the attitude that certain matters of opinion or judgement are matters of settled fact. A quick glide through classical test theory and reliability has been "done": now on to the serious business of discussing the value of ink-blots. As a result we have the all too common treatment of tests (referred to airily by acronym — a sure sign of the initiate) as the subject matter of psychometrics. This is the very opposite of the independent critical outlook one would wish to engender in those who may come to make use of tests. They may forget that those tested are people, and that test data do not have an independent existence apart from those people.

Non-American readers will find the list of tests considered parochial and occasionally out of date. They will also find the price beyond what it is reasonable to ask a student to pay. □

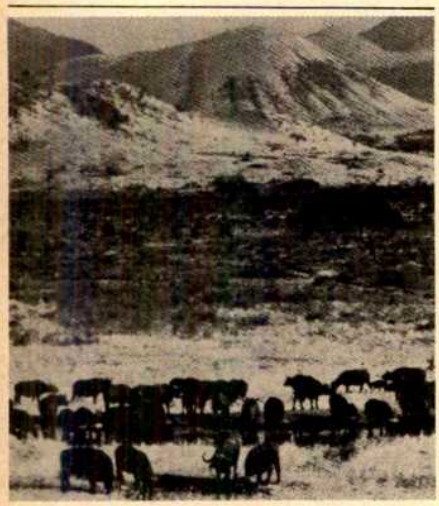
Steve Blinkhorn is Director of the Psychometric Research Unit and Senior Lecturer in Psychology at Hatfield Polytechnic, Hertfordshire.

Different behaviour

Richard J. Cowie

Animal Behavior: An Evolutionary Approach, 3rd Edn.
By John Alcock.
Sinauer/Blackwell Scientific: 1984. Pp.596. $25, £19.80.
Biology of Animal Behavior.
By James W. Grier.
Times Mirror/Mosby/Blackwell Scientific: 1984. Pp.693. $28.95, £32.50.

WITH new textbooks on animal behaviour appearing every few months, it is an achievement for any one of them to reach a second edition. John Alcock has gone one better, and his well established textbook is now appearing for the third time. What is more, revision of the previous edition has not just been cosmetic. The book has been largely rewritten and the material has been reorganized into a more logical sequence. John Alcock is a good writer, and like its predecessors this edition is a joy to read. It is also well produced, and the layout, figures, photographs and binding are superb. A nice addition is a series of colour photographs on the inside of the covers.



Buffalo at home on the range in the Tsavo National Park, Kenya. The picture is reproduced from Ecology of Natural Resources *by François Ramade, published by Wiley.*

Major changes to the content are the inclusion of a chapter on the development of behaviour and expansion of the section on sexual selection and mating systems. Both are improvements, but the book still has weaknesses which mar its usefulness as a general introductory text. These weaknesses stem from Alcock's interest in the evolution or function of behaviour, at the expense of a causal approach. For example, there is no mention in the index of such key concepts as motivation, ritualization or habituation, and the important topic of classical learning theory is dealt with in two short paragraphs under the sub-heading of plastic developmental systems.

Besides these obvious omissions there are two further points which concern me. The first is Alcock's tendency to invent explanations in terms of evolutionary advantage for almost every behaviour mentioned, including many aspects of human behaviour. Whilst I recognize the temptation to do this, there is a danger that naive readers may accept these speculations as established fact. My second worry is Alcock's failure to present any of the more mathematical models which have been successfully used to predict how animals do behave. This is particularly true for the many optimality models, which the author praises for producing quantitative predictions (p.200) but fails to describe in any detail. However, despite these weaknesses — or perhaps because of them — I am sure the book will remain popular with many students.

Whereas Alcock's book is restricted in outlook but well organized and enjoyable to read, Grier's book is just the opposite. It is one of the most comprehensive textbooks on animal behaviour that I have seen, but the sheer volume of information and the order in which it is presented leaves one a little breathless and bemused. That is not to say that the book is badly written and in general Grier's summary of each topic is good. He tackles many controversial subjects, such as the sociobiology debate, in an informed and balanced way.

Besides covering the more usual subjects, the author provides a crash course in both molecular and Mendelian genetics, an interesting historical perspective to the study of behaviour, and chapters on the observation and measurement of behaviour, abnormal behaviour, and applied behaviour, although these last two chapters are too simplistic to be of much use. The book is well laid out with many photographs and figures, the latter being generally well explained with lengthy captions, although some are confusing (for example those on p.42 and 43). There are numerous small details in which I would disagree with Grier, but overall I was very impressed with his encyclopaedic treatment of the subject and his evident grasp of many diverse areas of zoology.

If I were to choose one of these books to sit down and read for my own enjoyment, it would be Alcock's. But if I had to recommend one as a textbook to students it would be Grier's. □

Richard J. Cowie is a Lecturer in the Department of Zoology at University College, Cardiff.

Anything is possible

P.N.R. Usherwood

Neuroethology.
By Jeffrey M. Camhi.
Sinauer/Blackwell Scientific: 1984. Pp.416. $30, £24.

THE TASK of keeping abreast of developments in the biological sciences grows daily, even within the strict confines of one's own specialism. It was with some misgivings, therefore, that I approached this review, especially since my only credential was a brief love affair with invertebrate neuroethology in the late 1960s. However, my fears were unfounded since here is a book which is both highly informative and thought-provoking. Its aim is to introduce readers to the major concepts and research strategies which characterize neuroethology, a field of biology which continues to struggle for identity in an uncompromisingly compartmentalized world. Neuroethology remains an essentially schizophrenic experience, spanning neurobiology and ethology and making brave efforts to marry their languages and dogmas, but regrettably achieving little more, so far, than the connection of their fringes.

Neuroethology is essentially a book of two parts, one an introduction to the field, the other an account of nervous integration and behaviour. It is best when the author draws from personal research experience, one highlight being his description of cockroach escape behaviour and its neurobiological basis. My only criticism is that the emphasis is strongly anatomical and phenomenological with inadequate attention given to the advances in molecular neurobiology which have illuminated the past decade. There are good chapters on the visual and auditory worlds of animals, which contain essential background on physical factors in the environment, and useful accounts of sensory processing and motor control. Each chapter is concluded by questions for discussion and a good list of articles for further reading.

The author finishes, quite rightly, by marvelling at the complexity which characterizes the nervous systems of even "simple" animals and suggests, for example, that even after examining the circuit diagram of neurones in the leech we are left wondering how it works. This injection of mystery hints at a latent vitalism which denies the essentially mechanistic nature of neuroethology. He also lists six principles of neuroethology which he suggests have already been established and then argues that these are probably not global but restrictive and conditional. This lack of unifying principles is understandable in a fledgling science but it is unlikely to encourage the meek to enter the field. But Camhi has rekindled my interest in an old flame and all biologists with an interest in animal behaviour and nervous systems, simple and complex, will find his book well worth reading. □

P.N.R. Usherwood is Professor of Zoology at the University of Nottingham.

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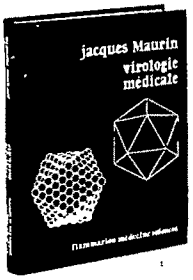
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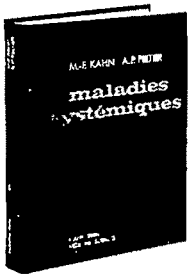


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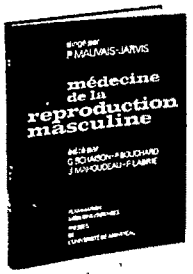
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Fresh selection

T.R. Birkhead

Behavioural Ecology: An Evolutionary Approach, 2nd Edn.
Edited by J.R. Krebs and N.B. Davies.
Blackwell Scientific/Sinauer: 1984. Pp.493. Hbk £25, $42; pbk £13.80, $25.

BEHAVIOURAL ecology has come a long way since the first edition of this book appeared six years ago, and is still moving fast. Some second editions are gentle updates, or ever so subtly improved versions of the first, but this is virtually a new book. There are very few chapters unchanged from the 1978 edition, the only exception being Maynard Smith's sex. Several of the chapter titles are the same as previously but often with new authors and certainly with a great deal of new information. As before the emphasis is on theory and the book is aimed at researchers and dedicated final-year students.

Behavioural ecology was born out of the cross-fertilization of ideas from several disciplines and it is exciting to find that the scope of *Behavioural Ecology* has expanded to include two additional topics, learning (Chapter 7 by Shettleworth) and plants (Chapter 14 by Charnov). While plants clearly don't behave as animals do, behavioural ecology theory has much to offer botanists. Considering the rich rewards that so many zoologists have gained from the use of selection thinking, it is somewhat surprising that botanists have lagged behind. Charnov's chapter deals with sex choice, breeding systems and the conflicting interests of males and females. These are not the only areas of common ground for zoologists and botanists; others (at Sheffield and the University of East Anglia) are already looking at the foraging behaviour of plants!

In Chapter 12 Emlen updates his review of cooperative breeding — clearly a necessity if the number of birds known to breed in this way is any measure of this subject's interest. In the first edition Emlen noted that there were 150 known cooperatively breeding birds: the figure is now 300! The diversity of such breeding systems has also increased but at a much faster rate than our understanding of how they evolved. Cases in which close kin act as helpers are relatively easily explained, but why unrelated individuals should help is more problematical. Emlen discusses reciprocity, but another possibility, not yet fully explored, is that helpers mate with breeding females and actually help to rear their own young.

All in all this is an excellent text. It is well produced, clearly written and provides plenty to think about. □

T.R. Birkhead is a Lecturer in Zoology at the University of Sheffield.

Wholesome ecology

Robert H. Smith

Ecology: Principles and Practice.
By W.H. Dowdeswell.
Heinemann Educational: 1984. Pp.312. Pbk £7.50.
Concepts of Ecology, 3rd Edn.
By Edward J. Kormondy.
Prentice-Hall: 1984. Pp.298. Hbk $29.65, £27; pbk $22.90, £20.85.
Principles of Ecology.
By R.J. Putman and S.D. Wratten.
University of California Press/Croom Helm: 1984. Pp.388. Hbk $22.50, £22.50; pbk $9.95, £9.95.

FEW textbooks that attempt to encompass the whole of ecology have received widespread acclaim. Odum's *Fundamentals of Ecology* (Saunders, 1953), Krebs' *Ecology* (Harper & Row, 1972) and Ricklefs' *Ecology* (Nelson, 1973) are the three which have been most often adopted as set texts, and all have run to second and third editions. Recently, the trend among publishers has been to launch series of smaller books, each dealing with a specialized aspect of ecology. However, many lecturers like to recommend a single book for purchase by first-year students which will also provide an introduction to later, more specialized courses.

Of the three books reviewed here, two are aimed firmly at the undergraduate market while Dowdeswell's *Ecology* is intended mainly for sixth formers and first-year university students. Dowdeswell's book is quite different from the other two and I shall consider it first. Dowdeswell provides a very basic introduction to ecological principles, going from physical factors through population dynamics to communities and ecosystems in less than 60 pages. No topic is considered in depth, nor is it intended to be. The book is written as extended notes with keywords highlighted in bold type and is clearly aimed at British, pre-university examination syllabuses. The second quarter of the book is about collecting and analysing data, and those involved in setting up simple field exercises for students may appreciate some of the practical tips. I do, however, have severe worries about the oversimplified treatment of statistics; for example, the statement "in order to avoid sampling error, the whole population should be collected but this is obviously impossible" does nothing to help the student struggling with the concept of the infinite population from which a sample is taken. The rest of the book runs through six groups of ecological communities and concludes with the obligatory chapter on ecology and man. I do not imagine that the level of description will be adequate for any biology degree courses, but the book is nicely produced and will probably do well in the pre-university market at which it is aimed.

Kormondy's *Concepts of Ecology* is a middle-aged standard, first published in 1969 and possibly running to fat in an expanded third edition. My personal view is that his treatment of ecology is unexciting in both content and presentation. Most of the references are to books or general articles in *Science* and *Scientific American*; though adequate for first- and second-year undergraduate courses, final-year students need something more demanding. Just as Dowdeswell's book is parochially British, Kormondy's is parochially American and I could not find a single reference to European ecological journals: have *Oikos*, *Oecologia*, the journals of the British Ecological Society or *Nature* never published anything worth mentioning? Kormondy's approach is the standard one, dealing first with energy flow in ecosystems and biogeochemical cycles, then with population ecology and community organization, finishing off with human ecology.

Principles of Ecology by Putman and Wratten is the largest and most comprehensive of the three, with over 500 references, mostly to original papers. The order in which material is presented is the first feature that strikes the reader; after the usual introductory chapter on tolerance to abiotic factors and physiological and behavioural homeostasis, the authors launch straight into the ecological community as the unit of study. In some ways this unusual approach is a good one, since energetics and nutrient cycling are dealt with in an integrated way with species-abundance relations, community dynamics and succession. However, I did find that the links between different topics in the first four chapters are not made all that clear, and I reckon that students will have some difficulty in following the chapter on the niche without first thinking about intra- and interspecific competition (which come later). It is in the chapter on interspecific competition that the deficiencies of treating species interactions before single-species populations become most apparent. In order to develop a Lotka-Volterra model, exponential and logistic population growth models are skimmed through in a few lines, and the presentation of the conditions for coexistence is terrible.

Putman and Wratten place their chapter on population structure and analysis centrally — which of course means that the central concepts presented there are rather lost. The expanded repetition of exponential and logistic population models is again badly presented and likely to confirm the average biology undergraduate's prejudices about mathematical representation. A brief consideration of life tables leads into key factor analysis and the use of k-values but, like many authors, Putman and Wratten do not emphasize the direct link between the methods of analysis and implicitly assumed population models. Perhaps I am

being too critical of their coverage of topics with which I am most familiar, but I believe that it is extremely important to present quantitative concepts as clearly as possible without oversimplifying.

My final comment is about the way that evolutionary questions are dealt with in ecology textbooks. Kormondy hardly considers such questions, though he mentions natural selection when discussing biotic potential — and in my opinion gets it badly wrong when he says "To remain in the game, it appears that for a decreased value of r compensations have been achieved by natural selection in increased generation time". Putman and Wratten have chapters on evolution and adaptation, and coevolution. Again, I could raise objections to much of what is said, but the authors are to be commended for stressing the relationship between ecology and evolution — albeit rather late in the book. Theories of evolution and ecology have not been well fitted together in the past, but things look more promising now. In particular, I feel that life history theory forms an important link, and behavioural ecologists are at last considering the ecological consequences of their theories and tests of optimality. These are two of the most exciting developments in ecology in the 1980s, and one hopes it won't be too long before they reach the textbooks. □

Robert H. Smith is a Lecturer in Applied Zoology at the University of Reading.

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Living in salt waters

Michael M. Mullin

Biological Oceanographic Processes, 3rd Edn.
By T.R. Parsons, M. Takahashi and B. Hargrave.
Pergamon: 1984. Pp.330. Hbk £22, $40; pbk £10.95, $19.75.
Marine Ecological Processes.
By Ivan Valiela.
Springer-Verlag:1984. Pp.546. DM 118, $44.
Basic Marine Biology.
By A.A. Fincham.
Cambridge University Press: 1984. Pp.157. Hbk £20, $36; pbk £7.95, $14.95.
Marine Biology.
By H.V. Thurman and H.H. Webber.
Charles E. Merrill: 1984. Pp.446. $28.95, £14.95.

ONE criterion in assessing the usefulness of new textbooks on marine ecology is whether recent findings, such as the hydrothermal vent communities, or renascent subjects, such as bacterial and protozoan ecology, are treated but also kept in perspective. Also important is whether the book covers other aspects of contemporary marine science — plate tectonics, mesoscale oceanographic fronts and eddies, interannual variability in oceanic climate and so on — in explaining marine biological phenomena. Finally, issues in general ecology, such as the role of disturbance in structuring non-equilibrium communities or food-limitation versus predator-limitation of population sizes, should be surveyed.

In the most strictly oceanographical book of the four reviewed here, Parsons and colleagues retain the "food web" emphasis of their earlier editions. They explicitly ignore intertidal and coral communities (and hydrothermal vents), and do not discuss sampling equipment. About 30% of the references are new and there are some new illustrations, for example of plankton at fronts, planktonic life cycles, isotopic ratios and how these can reveal food sources, and effects of pollution. To my mind, justice is not done to some new work (for example microbial heterotrophy), and the chapters on plankton seem to have been less thoroughly revised than the chapter on benthos. The concluding chapter on pollution and fisheries has been enlarged the most, and contains cogent accounts of why these problems continue to defy easy solution.

In its detail, emphasis on processes and citation of original literature this is a text for quite advanced students. As such, comments on the mismatches between what we can (and do) measure and what we need to measure would strengthen the early chapters. The authors do not shun algebraic equations, though the level of mathematical treatment of different subjects is uneven. In the next edition, the authors could further emphasize processes by combining related subjects, such as secondary production or bacterial chemosynthesis, for plankton and benthos.

In his book, Ivan Valiela has done this admirably; each chapter presents the conceptual basis of an ecological process and examples from the water column, the benthos (including intertidal and estuarine habitats) or fresh water. Valiela summarizes physical or geological aspects only as related to specific ecological processes; his emphasis is very much on biotic interactions, and he discusses fisheries in this context rather than separately. He cites very recent studies, dares to speculate occasionally, and frequently indicates the observational and experimental evidence behind a generalization. The book is not easy reading, and supplementation by material from oceanographical sources may be desirable, but advanced students will find here a stimulating summary of modern marine ecology. I wished only for a fuller account of interannual variability in oceanic circulation and its effects on distributions.

Fincham writes for a general audience, and understandably devotes much of his book to marginal areas — the intertidal zone and estuaries — on which many human activities directly impinge and which are accessible to classes. There are useful diagrams of physical processes affecting biology (even sea-floor spreading) and many drawings of organisms, though several illustrations are superfluous. The focus is upon organisms and their interactions with the physical environment; more abstract concepts of food webs and communities are briefly treated as qualitative and static generalizations. There is the expected account of fisheries, but also unusual chapters on behaviour and biogeography which introduce such recent evolutionary terms as reproductive strategies, kin selection and altruism (though I believe these concepts are less representative of marine ecology than is, for example, the cycling of nutrients). There are good drawings and descriptions of equipment from a historical perspective, but Fincham does not elaborate upon the sources and strengths of evidence. The discussion of the intertidal zone, for instance, could easily have illustrated the value of manipulation by removal, transplantation or caging of organisms.

The diversity of illustration, ranging from sketches so crude as to be useless to a section of colour photographs, impressed me in the massive text by Thurman and Webber. There are also "study aids" — end-of-chapter exercises and appendixes, the latter including the metric system, taxonomy, a glossary *and* a dictionary of prefixes and suffixes. Over a hundred pages are devoted to organisms *per se*, and many aspects of the environment are

treated in similar detail, all without the mixed blessing of citations from the original literature. The authors introduce some very recent work, biological and otherwise, and also give a taste of how contemporary oceanography is done. Insertion of brief essays on special topics, including a couple of speculative interviews, varies the pace for the reader.

I continue to prefer the drier, academic style of Parsons *et al.* and Valiela. Students who are mature enough to appreciate the enormous wealth of information presented by Thurman and Webber should also be curious enough to check original sources for points of evidence occasionally, and should be willing to cope with concepts in a mathematical as well as a descriptive form. □

Michael M. Mullin is a Professor of Biological Oceanography at the Scripps Institution of Oceanography, La Jolla, California.

Ecological thoughts

John Lawton

Community Structure and the Niche.
By Paul S. Giller.
Chapman & Hall/Methuen: 1984. Pp.176. Pbk £5.95, $11.95.
Predation.
By Robert H. Taylor.
Chapman & Hall/Methuen: 1984. Pp.166. Hbk £17.50, $39.95; pbk £8.95, $17.95.

PREDATION and interspecific competition are key ecological processes, although there is currently profound disagreement about their relative importance in structuring communities. Both phenomena have also spawned a diffuse and specialized literature that students and teachers alike find hard to break into. There is therefore considerable scope for good, simple introductory texts on both topics.

Giller's book has as its main theme the organization of communities, in particular the processes that control the numbers and relative abundances of species. It ranges widely to include discussions on predation, species–area relationships and food webs, although the dominant theme throughout is the concept of the ecological niche, as conceived by G.E. Hutchinson, nurtured by R.H. MacArthur and subsequently embraced by a whole generation of ecologists. In contrast, Taylor focuses on two- or three-species interactions, with a clear emphasis on mathematical rigour, testable hypotheses and the continual interplay between theory and experiment. Only in the last, short, chapter does he look at predation in communities, drawing the conclusion that "students of whole communities will need to be satisfied with a descriptive approach to static . . . patterns" — more or less what we find in Giller's book.

In general terms, therefore, the books make an interesting pair, with the potential at least to introduce students to a wide range of ecological problems and ways of thinking. Both are written for second- or third-year undergraduates, and postgraduates, with Giller's perhaps more accessible to novices and Taylor's decidedly more interesting for research ecologists of any age.

Specific details aside, I have some personal doubts about whether *Community Structure and the Niche* truly catches the spirit of its subject matter in the mid-1980s. Giller undoubtedly tries to explain controversies, and to draw attention to the uncertainties, doubts and problems that abound in this area of ecology. But he still left me with the impression that in spirit and philosophy the book could have been written in 1975. Moreover, I frequently found myself disagreeing with his main conclusions and interpretations, whilst in certain places crucial literature was ignored. A number of other things about the book also reduce its value as a teaching text. The 326 references appear in citation order (as in a letter to *Nature*), making it very difficult to follow the work of particular authors. More seriously, I frequently felt that the text was so packed with hurried references that many ideas would be unintelligible to all but the initiated. This fear was confirmed when I tested several sections on a brighter than average tutorial group. They found them cryptic and baffling. A slightly longer text, with less frenzied, fleeting allusions to everything that might be relevant would undoubtedly have made a better book.

No such criticisms can be levelled at Taylor. He explains theory and experiment with a lucid, direct style. Predator–prey interactions, ranging from protozoa, through insects, to birds and mammals, are mainly described using differential equations, but not in a way that should put off students — the mathematics is not very hard, and is made more palatable by plenty of graphical interpretations. I was particularly impressed by Taylor's careful discussions of why ecologists find it useful to construct mathematical models, and by his repeated attempts to highlight problems and difficulties, both in the construction of tractable models and in their empirical tests. In short, *Predation* should instil in young ecologists a way of thinking about problems that will be valuable whatever ecological processes they go on to study.

Predation may or may not be more important than competition as a force structuring ecological communities. Perhaps the next generation of ecologists will find such comparisons, and questions of relative importance, boring. But while we are training them to decide, I have no doubt that *Predation* provides more insights and a better foundation than *Community Structure and the Niche.* □

John Lawton is a Reader in the Department of Biology at the University of York.

Southern histories

Barry Cox

Vertebrate Zoogeography and Evolution in Australasia.
Edited by Michael Archer and Georgina Clayton.
Hesperian Press, 65 Oats Street, Carlisle, 6101 Western Australia: 1984. Pp.1,202. A$55. Post and packing A$10 inside Australia, A$14 overseas.

THIS is not so much a coffee-table book as a coffee-table in itself (area 650 cm^2, weight 3kg). Sixty authors have contributed 80 papers; most are concerned with Australia, but 13 deal with New Guinea, New Zealand and Lord Howe Island.

For Australians interested in biogeography and evolution, the trouble is that most books on these subjects treat Australian examples as a sort of awkward appendage, tacked on as a divine afterthought to the rest of Creation — a sabbatical daydream in which evolutionary fagends (for example monotremes) or pseudo-mediaeval beasts (kangaroos, wombats, moas and so on) were conjured up, their bizarreness emphasizing the sturdy normality of Northern Hemisphere animals, and their almost total lack of an evolutionary history teasing the poor palaeontologist. Australian students, in particular, have long needed a book centred on their part of the world. Well, here it is.

The first 13 contributions provide the background information relevant to Australasia in such topics as plate tectonics and palaeogeography, ecological zoogeography, the history of the climate and flora of Australia, vicariance and dispersal biogeography, and systematics. Each of the following sections on fish, amphibians, reptiles, birds and mammals commences with an account of the general evolutionary history of the group and ends with a checklist of the Australian representatives. The remaining contributions (a few being reprints of original research papers) deal with aspects of the zoogeography, evolution, ecology or discovery of Australian members of the group, or with the effects of man upon them. The book contains many figures and half-tone illustrations, and a few colour plates. The text is sometimes lighthearted in tone, and is also enlivened by occasional cartoons.

Though the quality and depth is inevitably a little patchy, this is in general an excellent student textbook. Even adding A$14 for postage and packing, it still works out at about 0.04p per page. So students should buy it now, use it as a reference book until it's out of date — and then have a ready-made doorstop. □

Barry Cox is Head of the Department of Zoology at King's College, University of London.

Biotas in space and time

Carolyn M. Harrison

Biogeography: An Ecological and Evolutionary Approach, 4th Edn.
By C.B. Cox and P.D. Moore.
Blackwell Scientific: 1985. Pp.236. Hbk $19; pbk £9.80.
Themes in Biogeography.
Edited by J.A. Taylor.
Croom Helm: 1984. Pp.404. Hbk £25; pbk £12.95.

WHEN the first edition of Cox, Healey and Moore appeared in 1973, it quickly established itself as a popular text and successive editions have served to reinforce its appeal. In this new edition, the authors have retained the essential structure of the immediate predecessor, and have expanded two chapters to discuss more fully how the major patterns of biota emerged in the distant and recent past. They do this with the distinctive blend of description and analysis adopted in earlier editions and the student is left in no doubt about the difficulties of reconstructing palaeoenvironments and their associated biotas.

The evident emphasis on analysis of the spatial response of biota to plate-tectonic movement and climatic change will be welcomed by many, but it has been achieved at the expense of any real discussion of how plants and animals adapt to their changing environments. In earlier editions, a chapter on natural selection, inbreeding and adaptation formed an important functional link between the evolutionary and ecological perspectives that the book seeks to forge. The omission of these topics in the new edition is not unexpected, given the complexity of evolutionary and ecological genetics; nevertheless their absence merely serves to reinforce a retreat from the stress on ecology that can be detected in the earlier editions. The task of preserving a balance among differing views of biogeography is a challenging one and it is easy to be dismissive of the value of ecological studies for students who are interested in the history of distinctive floras and faunas. But, for those large numbers of biogeographers who are intrigued by the impact of human activities on the distribution and survival of biota when seen as resources and cultural symbols, an ecological perspective is just as fundamental as an evolutionary one. As a result students in biology and geology departments will be better served by this new edition than will their fellows in departments of geography and environmental studies.

The tensions provoked by the position of biogeography at the meeting point of several disciplines is nicely revealed through the 11 essays edited by J.A. Taylor. This volume is addressed to the advanced student and aims to provide a representative selection of traditional and emergent themes in biogeography, as seen by British researchers. The result is something of a potpourri. In these essays biogeography emerges as a multifaceted subject and as one to be approached in a variety of ways and with a range of analytical tools. Each essay stands on its own; most are scholarly, informative and up to date; some are idiosyncratic and a few are dull. Of more concern than the disparate nature of the assembled topics, however, is that here an opportunity has been missed. Many researchers will be disappointed to find that the chance to raise the level of debate about research priorities was not grasped more fully. Moreover, aspiring researchers will find little to help them decide which of the several methodologies or techniques available are the most appropriate for tackling particular problems. This is not to belittle any one of the individual contributions to the volume, but rather to point to the benefits that a more obvious and focused discussion of research developments would have brought.

Biogeography has experienced something of a renaissance in recent years and some of its popularity can be attributed to the well-deserved success of books such as Cox and Moore. I cannot help feeling however, that students who are inspired early on in their careers by these authors, will not find their enthusiasm fanned equally by a reading of Taylor's volume. And that is a pity. □

Carolyn M. Harrison is a Lecturer in the Department of Geography at University College London.

Change in population

Michael B. Usher

Population Biology: The Coevolution of Population Dynamics and Behavior.
By John Merritt Emlen.
Macmillan, New York/Collier Macmillan, London: 1984.*
Pp.547. £31.50.

HOW would most population biologists answer the question "how much mathematics should a student know?". Dr Emlen leaves the reader in no doubt since his book commences with a mathematical introduction, the "tools of the trade". The tools largely consist of matrix algebra, with, in addition, complex roots, variance and covariance, partial derivatives and the Taylor expansion. After this explanatory introduction it is odd that these mathematical concepts, especially matrices, are not made more of in the subsequent text.

Most of the topics that one would associate with a population dynamics textbook are covered — populations with and without age structure, predator–prey interactions, competition and multi-species interactions — though it is, perhaps, surprising that parasitoids and mutualism are hardly mentioned. The book starts to diverge from the usual text when a strong genetical flavour is introduced in chapters that look at the genetic structure of populations and analyse models developed to deal with population parameters, either at a single locus or at multiple loci. Fitness is considered, largely by posing questions that raise a multitude of other questions: the same may be said about clines in colour, and their possible relation to environmental gradients.

The basic material having been covered, the evolution of various traits of population dynamics is then analysed in the final seven chapters. These traits include population parameters, age structure, mating systems and competitive interactions. The account of the latter is particularly concerned with niche separation, and emphasizes the coefficients proposed for measuring the effect of one species on another. The chapter dealing with predator–prey relations reviews the evidence for adaptations of both prey and predator, and for their coevolution.

The subtitle is somewhat misleading, since "behavior" is so underplayed in the text and the emphasis is firmly upon genetics; a subtitle such as *The Integration of Population Dynamics and Population Genetics* would have been much more appropriate. The book itself is almost dauntingly large, with large pages of small type, but it does encompass most of the subject, including reviews (to 1982), and incorporates discussion of many recent concepts. Although the style of writing seldom makes the text particularly lucid, I suspect that I shall make good use of this book for reference. □

Michael B. Usher is Reader in Biology at the University of York.

*Where appropriate, the price of a book in both Britain and the United States is printed at the head of a review in *Nature*. Prices are confirmed shortly before publication of a review.

It is apparently the policy of Macmillan Publishing Co. Inc. of New York (an entirely different company from Macmillan in Britain, although, confusingly, Macmillan in Britain will from 1 May 1985 *distribute* Macmillan Inc. titles in Europe) not to divulge prices of their books over the telephone except on the authority of "the vice president and director of marketing".

Attempts to persuade Macmillan Inc. to make public the dollar prices of *Population Biology* and *Microbiology*, 4th Edn (see p. 49) have failed, and these prices therefore do not appear here. The company might consider whether this policy is in its own best interest or that of potential purchasers of its books.

New Journal

Biology and Philosophy

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The 1970's have seen fascinating and controversial advances in the biological sciences. One thinks, for instance, of methods for analyzing the basic molecular units of heredity; of proposals and clarifications of the appropriate methods of classifying organisms; of exciting new ideas about the nature of the fossil record and their implications for evolutionary mechanisms; and of ways in which the biological study of behavior has been extended, theoretically and experimentally, and of supposed implications for humankind. On top of these internal issues, there have been debates about biology in the world at large, for instance in education. These issues and more have made biology one of the most exhilirating areas of science, and have aroused in biologists and philosophers an awareness that there is need for meta-theoretical analysis, both about the very nature of biology (methodology and epistemology), and about its social implications (ethics).
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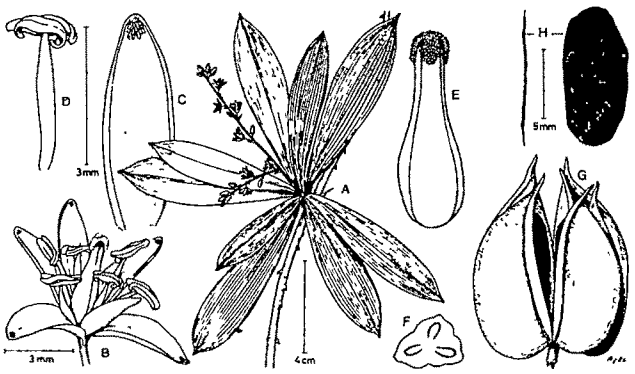
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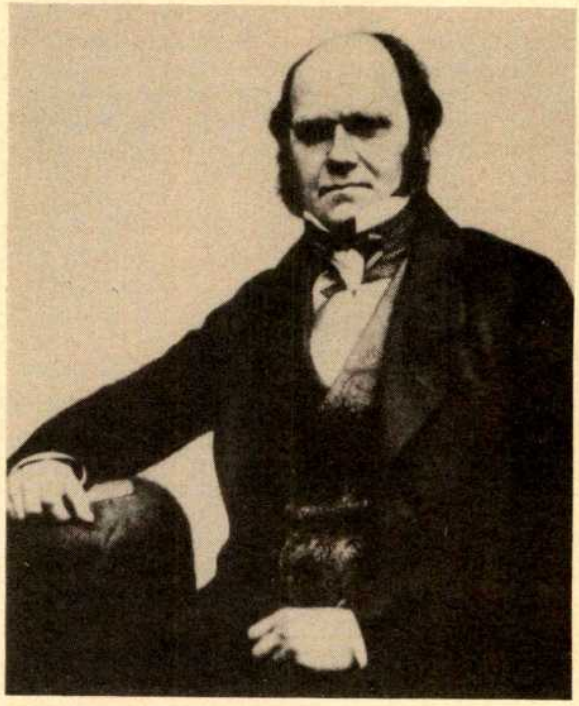
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Parts of phycology

Howard Pearson

Introduction to the Algae, 2nd Edn.
By Harold C. Bold and Michael J. Wynne.
Prentice-Hall: 1985. Pp.720. $53.95, £49.15.

UNDERGRADUATES requiring an up-to-date text on the algae, which concentrates on cell structure, reproduction and classification, will benefit greatly from this factual, well-written, concise and well-presented book. Researchers who spend most of their time "torturing" the physiology and biochemistry out of algal cells, but want to know how their "tame" species fit into the greater scheme of things, will also profit from reading it.

The book was not written to cover all aspects of modern phycology (a point acknowledged by the authors), but to complement other texts on algal ecology, physiology, biochemistry and genetics. In this respect it is not adequate for an introductory phycology course, or for students attracted primarily to the algae by an interest in their potential biotechnological applications.

This second edition retains the format of the first, with an outstanding introductory chapter (worthy in itself of publication as a booklet) preceding accounts of the algae division by division. The blue-green algae are included, as are new sections on *Prochloron* and algae of uncertain affinity. There are also other minor additions and changes to some of the keys. Mercifully, Bold and Wynne have dropped the unnecessary "phyco" from the algal division names so that, for example, the Chlorophycophyta revert to being the Chlorophyta. The glossary of terms is retained and aids the use of the simple-to-follow keys to the genera of each division, while the updated reference section provides a good lead into further reading. However, I regret the deletion in this edition of the very useful appendix on culture methods as the basic information greatly helps those embarking on the culture of algae.

In all this is an excellent book, full of useful pieces of information. The many photographs and line drawings complement the text and merit special praise. Bold and Wynne have managed to bring alive what even to many dedicated phycologists is a less than stimulating, albeit essential, area of the subject. □

Howard Pearson is a Lecturer in the Department of Botany at the University of Liverpool.

Eye to revolution

C.J. Leaver & S.M. Smith

Plant Molecular Biology.
By Donald Grierson and Simon Covey.
Blackie/Chapman & Hall: 1985. Pp.176. Hbk £17.95, $39.95; pbk £8.95, $17.95.

THE application of recombinant DNA techniques to the study of plant biology has led to the establishment of the new field of plant molecular biology. Yet it is only now that a book on the subject can be justified by the available data. In this short and up-to-date summary, Grierson and Covey have succeeded in conveying the present state of knowledge, as well as indicating the potential for genetic modification of higher plants which has raised hopes for a new green revolution.

The basic structures of plant genes are not dissimilar to those found in other organisms. However, the exciting questions in molecular terms arise from the presence in the plant cell of three interdependent genetic and protein-synthesizing systems, coupled with the unique developmental characteristics of higher plants.

The first chapter is a brief, obligatory introduction to recombinant DNA technology, which like the rest of the book would have benefited from the inclusion of a glossary to explain some of the necessary jargon. Subsequent chapters deal with the structure, organization and expression of nuclear, chloroplast and mitochondrial genomes. The chapter on hormonal and environmental factors affecting gene expression reflects our level of ignorance in this important area, and is disappointing in its lack of coverage of some of the better understood aspects of the responses of plants to stress, such as heat shock and anaerobiosis. The accounts of plant–bacterial interaction in nitrogen fixation and in the natural gene transfer system of the *Agrobacterium* Ti plasmid are excellent introductions to each subject, as is that describing plant viruses, and lead logically into a final discussion of the prospects for genetic engineering of plants. This chapter illustrates the fact that although it is possible to list some of the goals for plant improvement, we still have little idea of the identity or mode of regulation of the genes we might modify or transfer.

The text contains errors, but this failing is partly compensated for by the up-to-date bibliography which allows ready access to original papers. The authors have produced a book which provides a solid basis for undergraduate courses on plant molecular biology, and which will also be useful to the inquisitive, postgraduate and the informed entrepreneur requiring scientific background on the investment potential of plant biotechnology. □

C.J. Leaver is Reader in Plant Molecular Biology and S.M. Smith is Lecturer in Plant Biotechnology in the Department of Botany, University of Edinburgh.

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Chloroplasts, new and improved

R.P.F. Gregory

Chloroplasts.
By J. Kenneth Hoober.
Plenum: 1984. Pp.280. Hbk $42.50, £40.38; pbk $19.95, £19.
Chloroplast Metabolism: The Structure and Function of Chloroplasts in Green Leaf Cells, revised edn.
By Barry Halliwell.
Oxford University Press: 1984. Pp. 259. Hbk $50; pbk £9.95, $15.95.

IN THESE difficult times, when there is pressure to deliver courses that get to the point and stick to it, what usually gets left out is the sense of historical development of the present-day ideas, and all except the most sketchy treatment of comparative biology. It is a delight to find Hoober's text affirming at the very start his historical perspectives in a survey of the early stages of microscopy, chemistry and physics, and offering a challenging overview of the diversity of chloroplasts in algae as well as in higher plants. The work is well illustrated, with well-produced electron micrographs and line drawings. Equally welcome is the reappearance in paperback form of Halliwell's monograph; the reduced price will make this a more realistic prospect for the undergraduate.

The two books start off covering much the same ground, and present competent accounts of the structures, components and processes that make up the "light" and "dark" parts of photosynthesis. They also include practical details on such matters as chloroplast isolation which are likely to be found valuable by researchers and teachers.

From then on each author follows his personal interests. Halliwell is very strong on the interrelationships of photosynthetic metabolism, discussing at length (and with his practical interest) the pathways of C_3, C_4 and CAM photosynthesis and their control, the roles of the chloroplast envelope and of its shuttle systems, and providing discussions of oxygen toxicity and polyphenol production. Hoober on the other hand presents a major coverage of his speciality of chloroplast genetics and protein synthesis, including the difficult questions of the cooperation between the genomes and protein synthesis of nucleus and chloroplast (topics which Halliwell chooses to leave out except for a very brief note as an appendix). It is of course traditional to distinguish genetics and protein synthesis from metabolism, but this tradition is rapidly dissolving as the DNA workers prove themselves increasingly able to solve metabolic questions. Hoober concludes with a discussion of the evolutionary relationships of organelles and bacteria, and describes in some detail (and with further challenging questions) the work in progress on relating common sequences in proteins and in DNA, and so he returns to his initial theme of unity and diversity in this microcosm of Nature.

Halliwell's publisher is perhaps taking some licence in terming this a revised edition: there is in fact virtually no change of any substance (the pagination nowhere differs by more than one page from the original edition), and indeed Halliwell claims only to have made corrections and a few updates. He has provided some additional references to the literature, chiefly in the sections describing the Calvin cycle and photorespiration, but he has not incorporated the recent advances in (for example) the oxygen-evolving system, nor in the chloroplast genome. Nevertheless within its terms of reference Halliwell's book still stands up well to comparison: for example, on the question of the transport of metabolites across membranes, he indexes both the C_3 translocator and the dicarboxylate translocator, and discusses their roles in the export of NADPH and ATP. By contrast Hoober is very brief here, referring only to the phosphate translocator in connection with triose phosphate export. Such key terms, including also transketolase and the names of important metabolites and enzymes, are printed in Hoober's text in bold type, but surprisingly do not always appear in the index.

Both books clearly have their strengths, and will be needed by the teacher of biology, biochemistry and botany. The student who is unwilling to purchase both will have to judge carefully where his interests lie. □

R.P.F. Gregory is Senior Lecturer in Biochemistry at the University of Manchester.

Spring books

The next review supplement to appear in *Nature* is Spring books on 25 April.

The books to be reviewed include *Kapitza, Rutherford, and the Kremlin* (by Lawrence Badash); *The Problems of Evolution* (by Mark Ridley); *Time's Arrows* (by Richard Morris); *The Beginnings of the Nobel Institution* (by Elizabeth Crawford); *State of the World 1985* (by Lester R. Brown *et al.*); *The Broken Brain* (by Nancy Andreason); *Nuclear Winter* (by Mark Harwell); and *"Surely You're Joking Mr Feynman!"* (by Richard Feynman).

Green workings

J.D. Ross

The Physiology of Flowering Plants: Their Growth and Development, 3rd Edn.
By H.E. Street and Helgi Öpik.
Edward Arnold: 1984. Pp.279. Pbk £9.95.
Advanced Plant Physiology.
Edited by Malcolm B. Wilkins.
Pitman: 1984. Pp.514. Hbk £17.50, $17.50; pbk £14.95, $14.95.
Class Experiments in Plant Physiology.
By Hans Meidner.
George Allen & Unwin: 1984. Pp.169. Hbk £20, $35; pbk £9.95, $15.95.

WHEN they come to buy or use a book, the novice, the advanced student and the teacher have very different requirements. The beginner needs a broad coherent text which also brings out the excitement of the subject. The advanced student needs a detailed, balanced exposition and a lead into the current literature; whilst the teacher needs reassurance, a stimulus, and often a little help from his friends.

Perhaps the most difficult book to write well is the first, the "easy" one for the new student. In this third edition of *The Physiology of Flowering Plants* Helgi Öpik has based her text closely on successful earlier editions and concepts developed with her co-author, the late Professor Street. With minor flaws, she has succeeded admirably. The book flows through the subject with a nice style and conveys enthusiasm without avoiding uncertainties and controversies. Starting logically with seed imbibition and germination we are quickly introduced to the interrelations of axis and storage tissues. The accepted dogma is outlined and then the criticisms are aired, for which the author must be given credit and respect for her feeling that the initiate should be exposed to problem areas. With emphasis on whole-plant physiology, we are led through photosynthesis, water relations and nutritional aspects in clearly written chapters.

An interesting account of stress physiology is then followed by discussion of the various aspects of growth and development. Again difficulties are not ignored; for example on page 184 there is a section headed "The problem of the mode of hormone action" (other texts do not even admit to a problem). This is exactly the treatment needed by the new undergraduate; it will inform and interest. Sadly, the diagrams and figures are small and mean, a major fault in many British publications.

In the preface to *Advanced Plant Physiology*, Professor Wilkins gives his opinion that a cooperative effort is necessary in compiling a text for the advanced student. This approach indeed gives strength in one sense. Unfortunately it is also the root of many of the book's weaknesses and the 20 contributions vary widely in treatment and usefulness. From my differing degree of enjoyment in reading the various parts I suspect that Wilkins with, perhaps, his colleague John Hillman could have written a more acceptable book on their own. Chapters such as theirs, on gravitropism and apical dominance, successfully put across ideas and experimental approaches in a manner suitable for their intended audience. It is at

the student, not the researcher, that this text is aimed and balanced coverage is vital. This is not achieved in many of the contributions, although the editorial choice of subject divisions makes such a failing unavoidable.

The opening five chapters unashamedly tackle the "hormones", largely in a biased and sometimes dogmatic manner. The material contained here may indeed be recent, but the philosophy is still that of Frits Went. These accounts are not without scholarship, some being good reviews, but they deal with relatively narrow areas often of marginal relevance to normal plant growth. Also there are slips in terminology which annoy rather than confuse (although some are misleading, such as the use of "vernalization" for "stratification"; some plants can be vernalized as a seed, but this is a very different phenomenon to dormancy breakage).

I did not greatly enjoy this part of the book but found the rest of it more satisfying, albeit with some exceptions. The discussions of growth movements, rhythms, water relations and mineral nutrition are well constructed. Photosynthesis, however, is covered in a peculiarly unbalanced manner with aspects as important as C_4 metabolism and photorespiration being squeezed into the last two pages. Another odd component is the chapter on phytochrome — the undergraduate basing his studies on this is going to need guidance as, I suspect, will many lecturers. If this chapter is meant to be balanced by that following, on photomorphogenesis, it fails; here are the views, and largely the work, of one laboratory with little regard for other opinions. In contrast, the rather neglected phenomena of juvenility, photoperiodism and vernalization are well described and reviewed in a readable fashion, and are followed by straightforward accounts of germination, senescence and abscission.

Parts of this book are very good. But those using it must be supported by teachers unafraid to dispute the written word, so readily accepted by students.

Hans Meidner has written *Class Experiments in Plant Physiology* for those who teach the subject. Over the years a number of laboratory guides in plant physiology have appeared, usually emanating from the United States and written for students. These have not proved popular in Britain where there are smaller classes and different pressures. This time the collected experience of successful teachers and the author's attention to detail will be gratefully received. Class experiments in plant physiology, within the constraints of the timetable, are notoriously difficult. The suggestions, advice and hints contained here will be of great use to both the experienced practitioner and the new lecturer — if there are any out there in these hard times! □

J.D. Ross is a Lecturer in the Department of Botany at the University of Reading.

Membrane biology moves on

M.J. Selwyn

Membranes and their Cellular Functions, 3rd Edn.
By J.B. Finean, R. Coleman and R.H. Michell.
Blackwell Scientific: 1984. Pp.227. Hbk $19.50; pbk £9.80.

MEMBRANE biology has advanced rapidly in the five years since the appearance of the second edition of *Membranes and their Cellular Functions*, a book which covers a course in membrane biology at first- or second-year university level more completely and satisfactorily than most of its competitors. A revised edition is therefore both welcome and timely.

Much of the material is included in that excellent tome *Molecular Biology of the Cell* by Bruce Alberts *et al.*, but this is less convenient because of its weight and because the information is scattered through the book. Robertson's *The Lively Membranes* is stimulating but very much slanted towards bioenergetics, while Harrison and Lunt's *Biological Membranes* or Houslay and Stanley's *Dynamics of Biological Membranes* are pitched at a more advanced level.

This edition has grown in length and content, and the need for growth can be readily appreciated by comparing the chapters "Recognition, Response and Communication", "Secretion, Endocytosis and Lysosomes" and "Biosynthesis and Turnover" with their counterparts in the second edition. Together with a new chapter on membrane variations and membrane pathology, these are where the most expansion has been made, to include, for example, extensive descriptions of receptor mechanisms and the biosynthesis and processing of proteins which are incorporated into or pass through membranes. The chapters on membrane structure, and composition, and on ion and solute movements have also been thoroughly up-dated, but the treatment of the physical chemistry of ionic equilibria still lacks rigour.

The authors' style is readable and the layout — a central text with wide margins containing topic titles on one side, diagrams and equations on the other — makes for easy reading, browsing or reference. Overall this is an excellent volume, accurately written and attractively presented.

M.J. Selwyn is Dean and Senior Lecturer in the School of Biological Sciences, University of East Anglia.

To start with . . .

Roger Hill

Genesis on Planet Earth: The Search for Life's Beginning, 2nd Edn.
By William Day.
Yale University Press: 1984. Pp.299. Hbk $49, £35; pbk $14.95, £12.95.

THERE is no better example of the inadequacy of a multidisciplinary approach to an interdisciplinary problem than in the search for probable origins of life. Progress depends as much on the breadth of understanding and synthetic abilities of individuals as it does on the design of relevant experiments or on sagacious observation. Such qualities are demanded in equal measure from commentators on the field, and William Day is clearly competent to provide us with a useful review of current results, theories and speculations.

The conceptual dimensions of the subject call for an iterative development, and the repetition of subject matter in new contexts is a welcome feature of the book. For example, Day has chosen the fossil record as his first substantive topic, and the prokaryote-eukaryote distinction introduced here is fully described again after much compressed information on the cellular and molecular basis of life. After two chapters on bioenergetics, the reader is ready for a scientific presentation of the problem, and a review of prebiotic syntheses of present-day biomolecules follows. The crux of the matter, the interface between prebiotic chemistry and biochemistry, is seen to be the abiotic polymerization of preformed nucleotides. Encapsulation by lipid bilayers and rudimentary photosynthesis gives life in slow motion before the development of protein synthesis and the appearance of enzymes. Extraterrestrial life is considered briefly, and the twenty-seventh of 28 short chapters is devoted to the organic analysis of meteorites.

Inevitably, subject specialists will find much to disagree with in the detail of this wide-ranging review, and many chemical evolutionists will regard as implausible the claim that not only are present-day polynucleotides essential prerequisites for life of any kind, but also that they could appear spontaneously as the outcome of a sequence of highly probable prebiotic syntheses. But there is much enjoyable and stimulating reading here. Day's historical and statistical perspectives, his descriptions of possible pre- and early-life scenarios and his speculations on the nature of future evolution are more than sufficient to encourage the reader to persist through the occasional conceptual thicket.

One caution to students: the author frequently presents conjectures in the style of factual statements which, though easing the narrative, calls for an assiduously critical presence of mind. □

Roger Hill is Reader in Chemistry at the Open University.

Our own inheritance

Ben Carritt

Human Genetics. By Elof Axel Carlson.
D.C. Heath: 1984. Pp.432. $27.95.
Patterns of Human Heredity: An Introduction to Human Genetics.
By James R. Brennan.
Prentice-Hall: 1984. Pp.340. $35, £31.90.

IT SHOULD not be imagined that a reviewer's heart beats faster with the arrival of a book based on a one-term course for non-science students. Moreover, chapter headings such as "Reflections on the Human Condition" (Carlson's first) fill the cynical and hard-bitten experimentalist with dread. And in Britain, where the contribution of science faculties to liberal studies teaching is negligible, it is tempting to dismiss such books as an irrelevancy. I was therefore pleasantly surprised by how much I enjoyed reading the first of the two reviewed here.

Carlson's *Human Genetics* is indeed intended as an introductory text for students with "no prior science course work", and the treatment of many important areas is therefore necessarily superficial. In some cases, additional depth is provided in the form of extensive legends to the excellent figures, and in others the details are contained in tables. At times, this device deprives the author of the space to explain his point with sufficient clarity: it also tends to give the impression that the transmission of scientific knowledge is not the primary purpose of the book. In a sense, it would be as well if this were the case, because viewed strictly as a genetics text it can be faulted. I thought, for example, that the coverage of chromosome abnormalities was too sketchy, and that there was too much developmental biology. Neither could I see the reason for inserting an account of radiation damage as the third chapter when mutation is not covered until much later on.

Yet this is an immensely readable book, which seems to revel in the opening of controversy where almost any kind of biological principle is involved. IQ testing, the genetics of skin colour, the genetic basis of race and the "eugenics" programme of the Nazis are obvious inclusions, but there are many others besides. I particularly enjoyed the nuggets of useful statistical information, included in tabular form, such as the changing patterns of mortality, the causes of mental retardation in mental patients and so on.

Patterns of Human Heredity, aimed at a similar audience, is very much more a genetics text. The structure of the book is logical, proceeding through cell structure, DNA and chromosomes to Mendel, and then on to genetic diseases, polygenes and population genetics. The later sections of the book are well handled, particularly those which are mathematically based, such as polygenes and population genetics. One wonders, however, whether they might not be too advanced for a readership which is considered to be in need of the sort of detailed description of human reproduction which appears earlier in the book.

I found Brennan's use of language annoying and sometimes difficult to understand, but this may be a personal or a national difference in usage. Both he and Carlson provide review questions and summaries for each chapter — useful learning aids. Brennan also gives answers to his less discursive questions, which is helpful, if repetitive. □

Ben Carritt is in the Medical Research Council's Human Biochemical Genetics Unit, London.

Genetic similarities

D.J. Cove

Genetics, 2nd Edn.
By Charlotte J. Avers.
Willard Grant/Wadsworth: 1984. Pp.644. $27.50, £13.95.
Genetics, 3rd Edn.
By Ursula Goodenough.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.894. Hbk £24.95, $37.95; pbk £13.95.

THIS is the third opportunity I have had to review the annual crop of introductory genetics textbooks for *Nature* (see *Nature* **289**, 702–704 (1981) and **295**, 476–477 (1982) for earlier reviews) and I will not repeat here in detail my views on how I believe genetics teaching should cope with the integration of the classical concepts of the subject with the explosion of knowledge that has taken place as a result of molecular analytical techniques. This year's books are new editions rather than new texts and so it would be surprising if either offered any radical change in its teaching strategy. Avers remains an example of the "Mendel before molecules" approach while Goodenough is a "Let's start with DNA" book. Although the two authors have adopted what at first sight appear to be completely different teaching strategies, these books illustrate clearly how such overall strategies are rather unimportant. Each contains a chapter on Mendelian inheritance and there is little difference between them. Both stick faithfully to history, introducing Mendelism with Mendel and so, like most other textbooks of genetics, the student is required to cope all at once not only with segregation but also with diploidy and dominance. Both texts give the initial impression that each gene is represented by only two alleles, one dominant to the other, although Avers introduces multiple alleles and incomplete dominance much sooner than Goodenough. In both books, Mendelian genetics is set in a sealed compartment with little reference to modern knowledge of gene structure or function.

Population and evolutionary genetics are also treated similarly in both texts. The subjects are dealt with last, in another compartment almost like an embarrassing appendix. Set against the excitement of recent advances in genetics at the molecular level, it is now often difficult to interest students in population and evolutionary genetics even though these topics remain of central importance to the subject. The integration of these topics into the general body of genetics teaching is therefore crucial.

As is to be expected, the most significant changes which these two new editions incorporate is in the area of molecular genetics and both include an up-to-date account of modern techniques for the handling and analysis of DNA and of the findings to which these new techniques have led. Here Goodenough, building on the strength of the molecular and microbial side of her earlier editions, has the edge.

The treatment by both texts of the regulation of the use of genetic information and of the genetic programming of development remains anecdotal. Perhaps the most important principle to emerge out of the study of the regulation of gene expression is that general principles are sparse. The diversity of regulatory mechanisms involved in the control of metabolism is not stressed enough in either book, and, in both, developmental regulation consists of the best stories told in an unrelated manner. Here Avers, perhaps because she has less space, achieves slightly more integration. Except of course in the treatment of Mendelian genetics, neither book gives much space to plants. Avers in her table of the DNA content of "representative eukaryotic genomes" includes no green plant!

As our knowledge of genetics becomes more detailed, the subject becomes more challenging to teach especially in introductory courses. Conventional approaches become less satisfactory and integration more difficult since there is so much to be integrated. I believe that it is now best to begin such a course with a fairly complete but largely uncritical review of our current state of knowledge and then to deal with the experimental evidence which supports this overview, at the same time building flesh onto the initial skeleton. Neither of these texts adopt this approach. Both in fact offer us the same recipe as before and although they include all the necessary new ingredients the flavour is not much changed. If you liked either of these books before you should like the new editions more. If you are a first time buyer, however, I do not think they are among the best buys. □

D J Cove is Professor in the Department of Genetics at the University of Leeds.

Cells and the single author

J.G. Edwards

Cell Biology, 2nd Edn.
By Gerald Karp.
McGraw-Hill: 1984. Pp.896. $35.95, £29.95.
Cells and Organelles, 3rd Edn.
By Eric Holtzman and Alex B. Novikoff.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.660. £24.95, $32.95.

THE acclaim which greeted the publication of *Molecular Biology of the Cell*, by Bruce Alberts and five other authors (Garland, 1983), was not solely a response to the excellence of its parts. As Joseph Gall pointed out in his review (*Nature* **302**, 637; 1983), the inclusion of material on development, the immune and nervous systems and special features of plants, pointed to ways in which the teaching of biology at the cellular and molecular levels might be better integrated. In comparison, the narrower horizons of earlier textbooks may seem to do less than justice to the essential unity of modern biology, so one looks at their new editions for compensatory virtues.

Karp's book shares with Alberts's a much stronger component of molecular genetics than used to be found in texts entitled *Cell Biology*, but despite its new cover photograph of a myogenic clone still lacks Alberts's strong foray into developmental biology. Where both Karp and Alberts cover the same ground, however, the former more often manages to give some insight into experimental origins. For example, Alberts will tell you how many ribosomal genes there are in a *Xenopus* oocyte, but not about the anucleolate mutant; that allolactose is the real inducer of the lactose proteins in *E. coli*, but not about the diploids which helped unravel the system. Karp sometimes also has the edge in significant details (not just on matters such as oncogenes, where the passage of two years naturally makes all the difference).

In most areas with which I am familiar the reliability seems high, although I am unhappy about the way in which Karp presents the Hayflick phenomenon, both by discussion and juxtaposition, as the result of defective cellular processes. A balanced account would surely include the idea of programmed cell-death, and thus make better sense of immortalizing mutations. Karp's micrographs and diagrams (the latter now in two colours) are excellent. The whole is an impressive achievement, which runs Alberts much closer than I had imagined possible and will strongly tempt those of us who share the author's view of the importance of experimental history for current understanding.

The shorter *Cells and Organelles* is closer in content to a traditional cytology textbook, and much gentler with the molecular aspects. Controversial areas get balanced, essay-like discussion and there are virtues of a reflective kind. You have to look in Part III of Alberts to understand Karp's cover picture, but only Holtzman and Novikoff point out that this revelation of the route to multinuclearity was one of the early triumphs of cell culture. Small unreliabilities disturb confidence, however: a diagram of skeletal muscle in which the muscle fibres are drawn to look like fibrils; another of a hepatocyte with empty space labelled for a microfilament; EGF named epithelial growth factor; pyruvate produced anaerobically from glucose. In the review copy a number of micrographs are much too dingy for clarity and impact, and some are patchily unsharp.

A reviewer should perhaps be wary of laying emphasis on how a textbook handles his own small favourite corner. Nevertheless, the time has surely come for better treatment than can be found in any of these volumes (including, surprisingly, Alberts) of the real progress which has been made towards identification of the molecules which join cells together. If any reader is busy revising another textbook, or wants to know about this topic for other reasons, my advice is to ignore most of the secondary literature, and give due attention to the strategy first adopted by Gerisch and also put to such good effect by Gerald Edelman and others. □

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DNA in sound and vision

E.J. Wood

The Techniques in Genetic Engineering Video Library.*
Series editor R. Williamson.
IRL Press: 1985. PAL in VHS and Betamax, £85, $170 each tape, £640, $1,280 the set. Supplementary charge for PAL in U-Matic and NTSC and SECAM standards conversions.

LAST year I reviewed the first videotape in this series (*Nature* **309**, 566; 1984). Now that the whole series is available, how do the programmes live up to the initial promise of that first tape?

Each of the seven subsequent programmes concentrates on a specific technique or a limited area, and lasts 20–30 minutes. After the introduction of the presenter ("an expert in this field") by Professor Williamson, there is a brief and generally clear account by the expert of *why* a particular technique is of value, and usually some graphics are used to demonstrate in detail how DNA etc. is cut up and manipulated. These individuals mostly do not look directly at the camera, which I found somewhat annoying, while the level of background noise in some of the sequences is unduly obtrusive at times. These sections move fairly rapidly and would not easily be absorbed by the student at the first sitting. But then the video can be "stop-framed" or replayed, until the details are clear. The graphics themselves are quite well done, although they cannot be said to use animation to its fullest extent, at least in the earlier tapes.

The tapes can be viewed in any order, although most people are likely to do cDNA cloning first. Several times demonstrations are repeated — for example, preparing DNA by ethanol precipitation, making cells competent by adding $CaCl_2$, electrophoresis on slab gels and baking nitrocellulose filters in an oven. Maybe each is appropriate at that particular juncture, but given the cost of the whole set of programmes so many repeats were perhaps unnecessary.

A more general, but more serious point, is that in places too much detail is given about a technique, so that the underlying message becomes obscured; for example, it cannot really matter very much that the buffer reservoir of a slab-gel apparatus holds 0.8 litre of buffer, and excessive attention is paid to the disposal of radioactive pipette tips. Still, the demonstrations of techniques are indeed on the whole of high quality: the only exception to this is the demonstration of the microforge, which is poor but probably technically difficult to show.

In order to benefit fully from the tapes students would have to work through each a number of times, stopping and starting as necessary, probably writing down the sequences in the graphics. At this level I feel they would learn quite well from the tapes alone. The prize for the best and clearest tape goes to *Oligonucleotides*, closely followed by *Microdissection and Microcloning*, while the worst missed opportunity was in animating nick translation which could have been done so much better. But this must have been a difficult project to mastermind, especially to judge how much to put in and in what detail, and overall the tapes do succeed in their aim of being a "self-paced teaching aid". □

* 1. *Nucleic Acids Techniques — An Overview;* 2. *Gene Analysis and Southern Blotting;* 3. *DNA Sequencing Using M13;* 4. *Gene Libraries;* 5. *Expression of Cloned Genes;* 6. *Oligonucleotides — Synthesis and Use;* 7. *In Vitro Mutagenesis and Use;* 8. *Microdissection and Microcloning.*

E.J. Wood is a Senior Lecturer in the Department of Biochemistry, University of Leeds.

Recombinant limits

S.D.M. Brown

An Introduction to Recombinant DNA.
By Alan E.H. Emery.
Wiley: 1984. Pp.223. Pbk £6.75, $17.95.

Genetic Engineering in Higher Organisms.
By J. Roger Warr.
Edward Arnold: 1984. Pp.58. Pbk £2.85.

The Genetic Code and Protein Biosynthesis, 2nd Edn.
By Brian F.C. Clark.
Edward Arnold: 1984. Pp.76. Pbk £3.20.

Bacteria, Plasmids, and Phages: An Introduction to Molecular Biology.
By E.C.C. Lin, Richard Goldstein and Michael Syvanen.
Harvard University Press: 1985. Pp.316. Hbk $35, £34.95; pbk $18.50, £18.50.

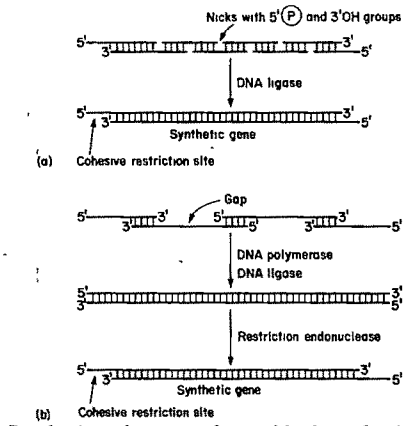
From Cells to Atoms: An Illustrated Introduction to Molecular Biology.
By Anthony R. Rees and Michael J.E. Sternberg.
Blackwell Scientific: 1984. Pp.94. Hbk £12, $12; pbk £6.80.

AS recombinant DNA techniques continue to stride forward there is an ever-growing movement to introduce this subject and its relationship to molecular biology at all levels of undergraduate teaching and in a wide variety of disciplines. There is also a strong demand for textbooks to cover the scope of the relevant techniques and the insights they have provided. But this need has disguised the fact that recombinant DNA is an "illustrator" of the systems it is used to investigate, and as a system *per se* has less intrinsic value; indeed, in many cases, the continued development of recombinant DNA techniques has depended upon the knowledge dissected from the biological systems under investigation. All this argues for an integrated approach to the teaching of recombinant DNA and genetic engineering in which the techniques cannot be divorced from the biology.

Nowhere are these dilemmas more painfully visible than in Alan Emery's *An Introduction to Recombinant DNA*. From the beginning Emery declares that he has not set out to write a textbook of molecular biology (of which, he adds, there are many good examples on the market). But, within a few pages, he is forced to explain DNA replication and subsequently, in the second chapter, genome organization has to be covered along with translation and transcription. The use of recombinant DNA techniques for the analysis of gene structure and function (Chapter 4) forces explanations of pseudogenes, transposons and multigene families until, at last, the author has lost the battle and the book reverts to a more integrated approach — describing various facets of recombinant DNA techniques within the context of molecular biology. Globin gene organization, immunoglobulin genes and oncogenes are all covered in following chapters, which discuss the molecular pathology of unifactorial genetic disorders and the molecular pathology of common diseases.

By now, it is apparent that the book is severely medically orientated and it continues in this vein. *An Introduction to Recombinant DNA* might more correctly be described as a textbook on molecular biology in medicine. This is not a thorough or careful account of recombinant DNA techniques — despite their probable importance in the future, some techniques such as embryo microinjection and the use of transgenic mice receive a most cursory mention — and the application of recombinant DNA in other disciplines is confined to a single, short chapter at the end of the book.

Roger Warr's *Genetic Engineering in Higher Organisms*, a volume in Edward Arnold's *Studies in Biology* series, concentrates on a wide range of higher systems and thus provides a more comprehensive coverage of methods for transferring genes into animal and plant cells. It therefore might seem to complement Emery's book. However, given the limited space available to the author within the pocket-book format of this series, the treatment of the subject is necessarily light and students will need to consult some of the texts provided in the reference list for a more detailed grasp of the systems described. In the same series, Brian Clark's *The Genetic Code and Protein Biosynthesis* has been revised and updated to provide what is almost the pocket version of Adams *et al.'s The Biochemistry of the Nucleic Acids* — a brief but clear and very historically orientated account of the elucidation of protein biosynthesis. Initially, I rebelled against the historical treatment, but the story illustrates the intermingling of biology and techniques that is the keenest approach to molecular biology. Still, some students, begging for final answers, may find this historical approach somewhat tortuous.



Synthesis of genes from blocks of oligonucleotides. The diagram is taken from Gene Cloning: The Mechanics of DNA Manipulation *by David M. Glover, published by Chapman & Hall/Methuen.*

Clark resists the temptation to introduce a mandatory chapter on recombinant DNA, but no such restraint has been shown by Lin, Goldstein and Syvanen in *Bacteria, Plasmids, and Phages.* The book is subtitled (in rather large letters, I might add) *An Introduction to Molecular Biology,* something that it patently isn't. This is a general introduction to microbiology with the emphasis on bacteria and its associated plasmids and phages. Bacterial genetics and genetic control systems, plasmid genetics, phage genetics and development are covered and there are cursory descriptions of some aspects of molecular biology, for example transcription, translation and allosteric enzymes. The book is very comprehensive but not especially detailed, so, for example, the decision pathway of lambda lysogeny is covered in a couple of pages. This is a basic primer for a bacteriology/virology course, with enough basic molecular biology thrown in to prevent the student's understanding of bacterial and viral systems from breaking down. A short chapter, "DNA Restriction and Gene Cloning", cannot make up for the absence of any discussion of the use of recombinant DNA in analysing bacterial or viral systems. Once again integration may have proved the better course.

A most refreshing aspect of Rees and Sternberg's book is that it does not claim to be anything it isn't. This is almost a coffee-table account of molecular biology, containing 44 sections dealing with topics from cell structure to gene organization. The structure of various macromolecules is covered in some sections and the book finishes with sections on muscle action, nerve action and the action of hormones and antibodies. Using a large-page format, each section takes up two facing pages and includes an extensive diagrammatic explanation which is accompanied by clear, concise notes illustrating the major points. The diagrams are of superb quality, managing to convey on the two-dimensional surface of the page, the four-dimensional dynamic quality of the processes they describe. Accompanying the diagrams a carefully measured text makes this a useful volume. However, it is not a textbook to be used in isolation — it will serve its purpose best as an adjunct to other more standard and comprehensive works.

If the measure of current textbooks on molecular biology, particularly with reference to recombinant DNA, may be taken from the present crop, then with one or two exceptions they seem generally of low standard. In part this stems from the apparent desire to be topical. But topicality alone cannot serve the interests of student or teacher in molecular biology. □

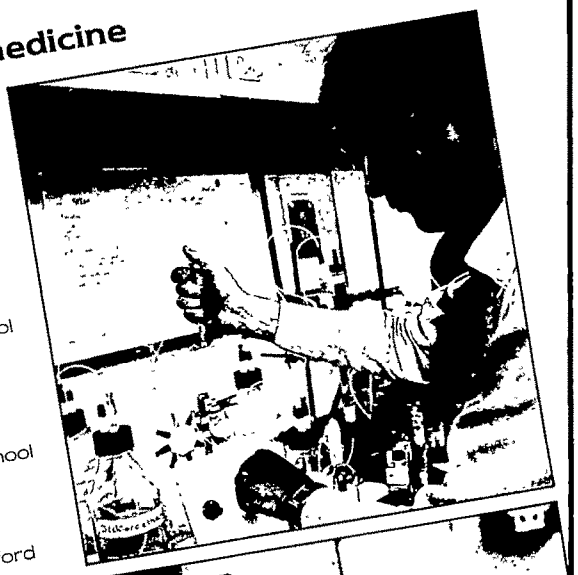
S.D.M. Brown is a Lecturer in the Department of Biochemistry at St Mary's Hospital Medical School, London.

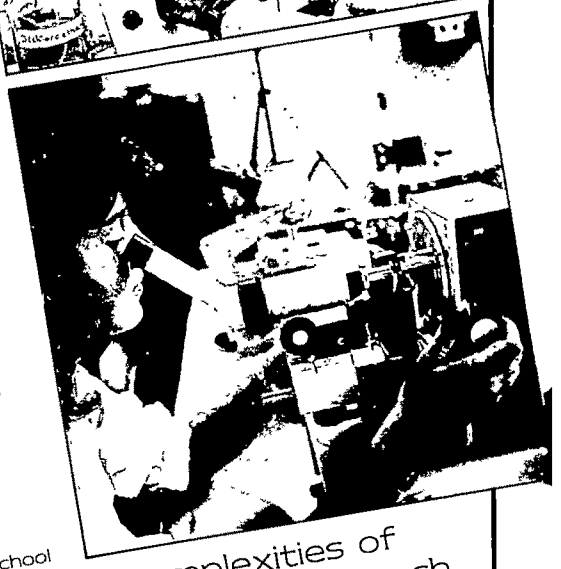
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Molecular defence

Andrew McMichael

Antibodies: Their Structure and Function.
By M.W. Steward.
Chapman & Hall/Methuen: 1984. Pp.96. Pbk £3.50, $7.50.
Introduction to Molecular Immunology, 2nd Edn.
By Alfred Nisonoff.
Sinauer/Blackwell Scientific: 1984. Pp.236. Pbk $14.95, £12.80.
Molecular Immunology: A Textbook.
Edited by M. Zouhair Atassi, Carel J. van Oss and Darryl R. Absolom.
Dekker: 1984. Pp.725. SwFr.129, $47.

IN THE past few years recombinant DNA technology has made a tremendous impact on our understanding of the immune response. This revolution is still accelerating as the T cell receptor, differentiation antigens and lymphokines are cloned and sequenced. Textbooks on "molecular immunology" appearing now — and written a year or two ago — are thus in danger of appearing outdated to those whose imagination is caught by today's excitement. At the time these three books were prepared, however, the new technology had been applied to antibodies and MHC antigens. So readers should not be disappointed, because these structures lie at the heart of the discipline.

The most concise and aptly titled of the three books is Steward's *Antibodies: Their Structure and Function*. The perspective is biochemical, with a clear and detailed account of the structure of immunoglobulins. The functional properties are discussed at the level of the antibody molecules, with equal attention being given to their ability to bind antigen at one end and to trigger secondary events at the other. The nature of the antigen binding is discussed particularly well and is the book's strongest point. Less attention is given to molecular genetics, although the essential features are covered. This is a solid and well written book, suitable for undergraduate biochemists or immunologists. It should serve as a good introductory text and has a plentiful bibliography.

Nisonoff's *Introduction to Molecular Immunology* is a superb book. It concentrates mainly on immunoglobulins and takes the reader carefully through the antibody molecule, from its binding properties to primary and three-dimensional structure and on to genetic control. Starting from first principles, including a brief description of the cellular background, it should be fully comprehensible to most biologists. The clarity of the text is not the result of over-simplification and the reader is taken up to the front line in research as it was in January 1984. The illustrations are good and always help to clarify the text.

This is the second edition of an already successful book and author and publisher are to be commended on the rapid appearance of the updated version. The new edition has been expanded by extensive rewriting of the chapter on complement and inclusion of a new chapter on the major histocompatibility complex. The latter is written with the same emphasis on biochemical and genetic information and has a brief introduction to explain why the MHC is important. This book will appeal to students at all levels, making an excellent introductory text for undergraduate courses and a useful revision text for those of us who have not followed this literature closely. I look forward to the third edition, hoping that new chapters on the T cell receptor, differentiation antigens and lymphokines will be added and explained with the same clarity.

Molecular Immunology, a multi-author work, is aimed at graduate courses and is much broader in scope than the two books discussed above. Unfortunately it seems to have taken longer to appear and recent advances make it look somewhat dated. The best parts of the book are the extensive (139-page) discussion of antigens and the following section on antibodies. The discussion of antigens is made up of several interesting chapters, although some have a rather narrow perspective — for instance, Atassi exclusively describes his own work on defining antigenic epitopes on myoglobin, lysozyme and haemoglobin — while the section on antibodies is less detailed than that found in the other two books. Fearon's chapter on complement, however, is one of the best short descriptions of this topic that I have seen.

The section on cellular immunology lacks molecular information and may seem to overemphasize phenomenology, but it should be realized that most of the new discoveries are confirming and explaining the observations of traditional immunology. There are several chapters on methodology, with a particularly good section on electron microscopy by M.A. Gonda (though I was surprised there was only a brief description of flow cytofluorometry in the account of cell separation techniques). The balance of this book is rather different to the other texts, and it should therefore form a useful addition to library collections. □

Andrew McMichael is a Professor in the Nuffield Department of Clinical Medicine at the John Radcliffe Hospital, Oxford.

Tackling immunity

Brian Solomon

Essentials of Immunology.
By W.H. Hildemann.
Elsevier: 1984. Pp.146. Dfl. 60, $19.50.
Immunology: An Introduction.
By Ian R. Tizard.
Holt Saunders/Saunders College: 1984. Hbk £21, $31.95; pbk £7.95.

CHOOSING an original title for a book on basic immunology must nowadays be a thankless task. Unfortunately, *Essentials of Immunology* is too similar to the title of the well-established *Essential Immunology*, by Ivan Roitt, which is very different in style. In his preface the late Bill Hildemann suggested an alternative title of *Immunology in a Nutshell* and after reading the book I consider that this would have been much more appropriate.

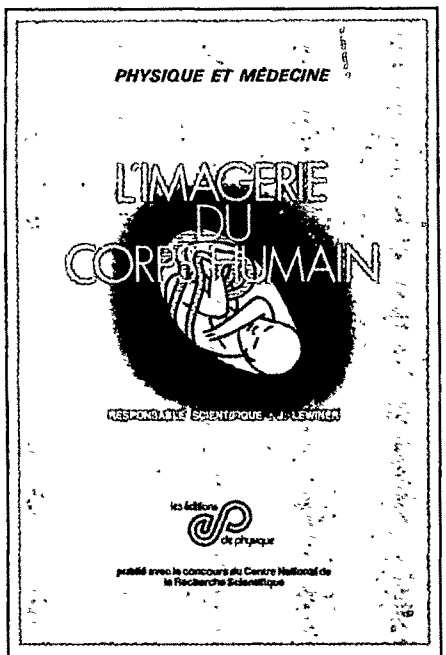
The book's stated origins lie in a lecture course for medical and science students in the health sciences. This probably explains why the text resembles lecture notes and there is a heavy reliance on diagrams and complex tables. There are some paragraphs where there is a true flow of words, but usually facts are given in a jerky note form and the reader is soon referred to a table or figure for more information. With such a sparse text, generalizations have had to be made, inevitably so, but some are dangerously misleading. For example, the process of opsonization is not "complement-dependent" (for IgG) but IgM will only function as an opsonin when bound with $\overline{C1423}$; the Chediak-Higashi syndrome is chiefly notable for a deficiency of natural killer cells; and the propensity for immature B cells to be driven into tolerance by antigen (a strong teaching point) is not even mentioned.

Although the book concentrates on human immunology there are sudden switches to mouse or chicken experiments without warning. This occurs particularly in the "Overviews" at the end of each chapter. For example, the statement "that fetal and newborn mammals acquire passive immunity by placental passage of IgG and by assimilation of sIgM and sIgA from the colostrum" is bound to confuse medical students.

As a cellular immunologist, the author regards immunoglobulins as "ancillary to cell-mediated immune functions", and the sheer magic of the widely different biological functions of the various immunoglobulin classes is lost in a massive table. There is only one paragraph on ontogeny, while considering Bill Hildemann's enormous contribution to research on immunorecognition in invertebrates it was also disappointing to find only two short paragraphs on phylogeny with one unnecessary table and another with misleading information. In short, I found too little nut inside the nutshell.

In Ian Tizard's *Immunology: An Introduction*, the reader is struck at once by the confidence of the author's writing. Many grey areas of my knowledge were dispersed by this book. At the same time, newcomers to immunology will find all their basic requirements for getting to grips with the subject. The opening chapters follow a

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conventional pathway through an early history of vaccines (Pasteur, Jenner) to antigens, major histocompatibility complex and phagocytes, followed by inflammation. Next, we suddenly leave cells for three chapters on antibodies before "tissues of the immune system" are approached. I feel a more logical order would have been to describe lymphoid organs and the differentiation of lymphocytes, then go on to discuss the induction of an immune response which would lead to a description of end products such as lymphokines and antibodies.

Among the antibodies much attention is given to allotypes, to the neglect of the sugars. Immunoglobulins are glycoproteins, and although the role of the oligosaccharides is still largely unknown they may help to dictate the various biological functions of the immunoglobulins. To indicate in a figure that IgE only has sugars bound to the C_H2 domain (like IgG) is incorrect as six oligosaccharides have been reported on three different C_H domains of IgE. There follows an excellent chapter on how to measure antigen–antibody reactions and antibody affinity, including a lucid section on antigen–antibody interaction with Fc receptors on cells.

As the author leads us into the jungle of immunoregulatory processes, he looks back at us over his shoulder to offer encouragement to "who has managed to follow so far"; we later graduate to a reader "who has managed to follow so far"! This is not because the book is difficult to understand, rather a sympathetic way of apologizing for the complexity of the subject. A stimulating chapter "Immunity at Body Surfaces" precedes another on vaccines which are dealt with very competently. However, absent here is an assessment of how successful vaccines have been in the improvements in public health from the turn of the century to the present day, and in the introduction of antibiotics. As vaccines are designed to induce memory cells and many properties of murine B memory cells have been described it was a pity not to include them.

The highlight and declared *modus operandi* of the book lies in the account of mechanisms of resistance to disease. This topic is bravely treated by separately dealing with bacteria, viruses, protozoa and helminths, an approach to a difficult subject which exposes many of the "nasties" practising immune evasion. A certain weakness in defining tropism with the microorganism's reliance on specific receptors on target cells can be excused as being "non-immunological", but I missed any account of the Duffy blood group – *Plasmodium vivax* story. Still, I like this book — it is how I would have described immunology myself and it has something in it for everyone. □

Brian Solomon is a Lecturer in the Department of Bacteriology at the University of Aberdeen.

Enzymology in the golden ages

Keith Tipton

Enzyme Structure and Mechanism, 2nd Edn.
By Alan Fersht.
W.H. Freeman: 1985. Pp.475. Hbk $24.95, £28.95; pbk £14.95.

ALTHOUGH an understanding of the behaviour of enzymes is essential for any full appreciation of the behaviour of living systems at the biochemical level, enzymology is too often regarded as being an esoteric and somewhat purposeless pursuit or a pedestrian and ossified technology. Anyone with such views should read this book. It is the author's contention that we are now entering a new golden age of enzymology, but this scholarly and enthusiastically written account suggests that such an age has existed for many years and will continue for many more.

A book of this size could not be expected to provide a comprehensive coverage of all aspects of enzymology and it is particularly strong in areas such as transient kinetics, enzyme mechanisms, and specificity and editing mechanisms, in which the author has been particularly active himself. However the generally well-chosen reference lists should allow readers to develop their knowledge in areas that receive less detailed coverage, for example steady-state kinetic analysis and the behaviour of metabolic pathways. The book is never dull and in controversial areas, such as the evolution of enzyme kinetic power, the author presents his own arguments cogently and convincingly.

Many will be familiar with the first edition of this work. In addition to the revision of much of the original material there are several new chapters, among them an account of enzyme stereospecificity, a topic which is often neglected in books of this type, and an excellent treatment of genetic engineering and enzymology. This latter chapter includes a particularly valuable consideration of the techniques of site-specific mutagenesis and their importance to the future development of the study of enzymes.

I have been recommending the first edition of this book to students, both undergraduate and graduate, since its publication in 1977. The appearance of this revised and expanded version is most welcome. I recommend it most highly as a stimulating and informative guide in which the ideas and principles behind our current understanding of enzymology are presented in such as way as to capture much of the excitement of the subject. □

Keith Tipton is Professor and Head of the Department of Biochemistry at Trinity College, Dublin.

Modern microbes

G.P.C. Salmond & R. Whittenbury

Biology of Microorganisms, 4th Edn.
By Thomas D. Brock, David W. Smith and Michael T. Madigan.
Prentice-Hall: 1984. Pp.847. Hbk $51.25, £46.70; pbk $26.95, £15.95.
Microbiology, 4th Edn.
By George A. Wistreich and Max D. Lechtman.
Macmillan, New York/Collier Macmillan, London: 1984. Pp.909. £40.*

"MAINLY old wine in new(ish) bottles" describes, perhaps unkindly, the two textbooks reviewed here. The first, a new edition of *Biology of Microorganisms,* has emerged with two new co-authors joining the originator of the enterprise, T.D. Brock. The product is a success, building on the previous tried and proved formula. Updating, particularly of the genetics and genetic engineering contributions is the major change from previous editions. Brock, the senior author, is widely regarded as the working general microbiologist's general microbiologist; consequently the content, style and depth of treatment, choice of illustrations, and table and diagram construction fit well with a general microbiologist's conception of what constitutes a good student textbook.

The microbe in its own right holds sway here, we are please to observe; for instance, accounts of structure, function, biochemistry and genetics, and of the microbe in relation to the environment, as a parasite and as a symbiont, all hang together in a way which draws the reader fairly painlessly into the complexity of modern microbiology. In all there are 19 chapters, with useful appendixes, a glossary and a reasonably comprehensive index. A particularly useful chapter is the last one, which describes representative prokaryotic groups and is, in effect, a series of compact essays conveying the essential biology and the unique attributes of each group. This book compares well with the other leaders in the field and is a strong contender for adoption by undergraduates reading courses in which microbiology is a significant component.

Wistreich and Lechtman's *Microbiology,* also in its fourth edition, represents a brave but, in our eyes, unsuccessful attempt to produce a modern balanced textbook of general microbiology. The book is organized into eight parts; the relative emphasis on the constituent subjects, however, is very uneven. For example Part II is designed to inform on the wide topics of growth, cultivation, metabolism and genetics. This covers only three chapters (6, 7 and 8) accounting for about 75 pages. On the other hand, medical microbiology and immunology start at Chapter 19 and go through to the end of Chapter 28, taking approximately half of the book. The rest of the book covers various matters, including microbial ecology, industrial microbiology, cell structure and function, and virology.

Perhaps worse than the imbalance in treatment is that the text is superficial in terms of content. Indeed, many parts of the book are likely to be totally misleading, either due to bad grammar or errors of fact. Chapter 8 on microbial genetics is a particularly illuminating example on both counts. It is littered with confusing information, contains mistakes and is badly out of date (for instance, no mention is made of yeast genetics). The poor balance of the book shows up even here where only four pages are dedicated to the topic of genetic manipulation — probably *the* cornerstone technique of modern biology!

There are some good points to the book, however. On the whole, it is easy to read, there is a wealth of diagrams in both black-and-white and colour, and the photographs are of a high standard. Each chapter begins by listing the things that you should know, and ends (in a form suspiciously like a list of examination questions) with a summary of the salient points plus questions and suggestions for further reading. Our impression, then, is that although this is an excellent microbiology "picture book" the very heavy emphasis on things medical severely perturbs its balance. □

G.P.C. Salmond is a Lecturer and R. Whittenbury a Professor in the Department of Biological Sciences at the University of Warwick.

*See footnote p.40

Bodily functions

Ole H. Petersen

Anatomy and Physiology, Vol.1, 2nd Edn.
By Edwin B. Steen and Ashley Montagu.
Barnes and Noble/Harper & Row: 1984. Pp.406. Pbk $6.95, £4.95.
Essentials of Human Anatomy and Physiology.
By Elaine Nicpon Marieb.
Addison-Wesley: 1984. Pp.355. Pbk $17.95, £18.
Physiology of the Human Body, 6th Edn.
By Arthur C. Guyton.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.691. Hbk £32.50, $37.95; pbk £8.50.
Essentials of Physiology, 2nd Edn.
By J.F. Lamb, C.G. Ingram, I.A. Johnston and R.M. Pitman.
Blackwell Scientific/C.V. Mosby: 1984. Pp.465. Hbk $18.95; pbk £12.50.

IN THE preface to Guyton's *Physiology of the Human Body* it is correctly stated that " . . . physiology continues in a dynamic stage of discovery with new knowledge of basic physiological concepts generated each day". Unfortunately, these new concepts do not figure prominently in any of the four textbooks reviewed here, and this is clearly due to the practice admitted in the preface to Lamb *et al.'s Essentials of Physiology*: "The reference list included is generally that which we found useful and usually consists of other secondary references, i.e. larger texts, monographs, etc". In other words, authors of physiology textbooks mostly get their information from other textbooks of physiology.

Steen and Montagu's *Anatomy and Physiology* is not a real textbook, but more a compendium of basic anatomical and physiological facts presented in a small-format paperback. It is in two volumes, but at present only Vol. 1 covering blood, circulation and the muscular and digestive systems is available. The index is not bad and when the companion volume appears this work could well be useful for last-minute revision purposes.

Essentials of Human Anatomy and Physiology is designed to meet the needs of students in the allied health fields and as such is in direct competition with the more attractive *Human Anatomy and Physiology* by Solomon and Davis which was reviewed last year (*Nature* **308**, 129; 1984). The anatomy sections are better than those dealing with physiology, in which basic errors can be found; for example the figure illustrating exchange of oxygen and carbon dioxide between tissues and capillaries indicates wrongly that the red cells are only concerned with oxygen and that all carbon dioxide is transported in the plasma.

Guyton's *Physiology of the Human Body*, now appearing in its sixth edition, is a thoroughly professional book with particularly good black-and-red diagrams and a text which is easy to read. It covers the same ground as *Textbook of Medical Physiology* by the same author, but with less emphasis on medical aspects. Each chapter starts with a brief overview summarizing the most important points and ends with a short list of questions and references. However, in spite of the addition of some new references, the book could essentially have been written 20 years ago. Key topics such as control of the heart, nerve action potential and transport in the kidney tubules are discussed without any reference to the important results obtained with the new methodologies of recent years. This can make the presentation unnecessarily complicated, when, for example, the electric action potential is explained in terms of ill-defined permeability changes instead of through opening and closure of specific ion channel molecules in the plasma membrane.

Unfortunately, Lamb *et al.'s Essentials of Physiology*, which is aimed at medical,

dental and science students, is only marginally better than Guyton's book in explaining the basic bioelectric phenomena which are central to so many physiological processes. Indeed one crucial figure (2.3 on p.29) is both confusing and incorrect by mixing up permeability and conductance, and the muddle is complete when megaohm is used as the unit! *Essentials of Physiology* is much more up to date than Guyton's textbook, but for a second edition it contains an astonishing number of omissions, mistakes and inconsistencies. Lundsgaard, my teacher as an undergraduate student in Copenhagen, often expressed the view that those unable to describe the effects of stimulating the sympathetic or parasympathetic nerves to the heart should not expect to survive his examination, but a present-day student relying on *Essentials of Physiology* would be in trouble as the positive inotropic action of sympathetic nerve stimulation is not mentioned explicitly in the section on the regulation of cardiac output. How marvellous if this and the other textbooks were to explain that this inotropic effect is due, at least in part, to an increased flow of calcium ions into the myocardial cells resulting from activation of selective calcium channels in the plasma membrane by the elevated intracellular cyclic adenosine monophosphate concentration (Reuter's excellent review of the process — *Nature* **301**, 569–574; 1983 — is recommended to all those writing a physiology textbook).

Lamb's book nevertheless has many positive features, such as the introductory ideas in the first chapter with its instructive and amusing figures, the excellent description of the mechanics of muscle contraction and the useful appendix on units and formulae. But one cannot help feeling that the authors have failed to read carefully and critically the whole text before sending it to the printers; thus the hormone cholecystokinin is variously referred to as CPZ, pancreozymin, CCK-PK, CCK and CCK-PZ, cyclic adenosine monophosphate is introduced as cyclic AMP without explaining the abbreviation and some of the diagrams (for example that of the exocrine pancreas on p. 155) are lacking in precision and likely to be misunderstood by many students.

None of these four textbooks provide good examples of how physiological data obtained at the cellular level can help us to understand important disease symptoms. The osmotic diuresis seen in diabetes mellitus, for example, can be simply and logically explained on the basis of the known transport characteristics of the different segments of the nephron, but none of the authors even mention the term osmotic diuresis. It is indeed a pity that many such good opportunities for helping students to use physiological reasoning have been missed. □

Ole H. Petersen is George Holt Professor of Physiology at the University of Liverpool.

The developing side of biology

Mary Bownes

Developmental Biology, 2nd Edn.
By Leon W. Browder.
Holt Saunders/Holt, Rinehart & Winston: 1984. Pp.748. Hbk £32.95, $37.95; pbk £17.95.
Developmental Control in Animals and Plants, 2nd Edn.
Edited by C.F. Graham and P.F. Wareing.
Blackwell Scientific: 1984. Pp.519. Hbk $37; pbk £18.50.
Genetics and Development.
By James H. Sang.
Longman: 1984. Pp.398. Pbk £13.95, $25.

THE rapid advances in our understanding of the genetic control of development and the molecular basis of developmental mechanisms make this an exciting period for students of developmental biology. All three of the books reviewed here cover both plants and animals, which is a major step forward, although in each case the space devoted to plant development is rather less than that devoted to animals.

Browder's *Developmental Biology*, now in its second edition, is the most basic text of the three and is extremely well written, up to date and enjoyable to read. The introduction provides a historical look at the topic, sets the context for the following five sections and briefly describes the main aspects of animal development. The subsequent sections each contain two to four chapters, advanced topics being dealt with in essays set aside from the main body of text. "The Cell in Development" (Section 2) covers not only the basic concepts of determination and differentiation but also chromatin and its structure, RNA and protein synthesis and the mechanisms of regulating gene expression, both transcriptional and post-transcriptional, and finally the cytoskeleton. This should give students a good idea of where many of the advances are being made, and the author provides sufficient background in molecular biology and genetics to make the section genuinely valuable rather than merely superficial.

There are detailed analyses of spermatogenesis, oogenesis and plant gametogenesis, followed by chapters on fertilization and "becoming multicellular". There is then a return from the more cellular experiments to the molecular, and the role of gene expression during the initiation of development is well described. The final section describes the processes of forming a multilayered embryo, and generating the wide range of cell types organized such that they create a functional organism. Throughout, the experimental basis of the subject is emphasized and well explained, and the text is beautifully illustrated with many clear and informative photographs and diagrams. This is a book that all students interested in development will find very rewarding.

The other two books are aimed at advanced undergraduates and, to some extent, at postgraduates and research workers too. Graham and Wareing's *Developmental Control in Animals and Plants*, also in its second edition, is a multi-author effort. The disadvantages of such volumes are well-known — lack of continuity of style and approach — yet, of course, in compensation each person is an expert in a given area. My expectation was that the book would thus be well up to date, but although the chapters are generally well written and interesting, only the final contribution by Woodland and Old ("Gene Expression in Animal Development") includes the kind of very recent experimental analysis one would hope for. This is fortunate since this area is moving especially fast.

The six parts cover developmental processes, the origin and maintenance of cell heterogeneity, cell communication, pattern and form, hormones and the molecular biology of development. One of the main aims of the authors was to present both plant and animal development at an advanced level. Several chapters in Part 5, "Hormones in Development", fall rather short of this. In the plant chapters there is no mention of the current controversy over plant growth factors, the authors taking a very classical approach to the topic which is perhaps no longer appropriate, while the chapter on hormones in animal development is somewhat narrow, concentrating rather too much on steroid hormones and the chick oestrogen and *Xenopus* vitellogenin systems.

Sang's *Genetics and Development*, intended for those interested in the genetic mechanisms controlling development, fills a long-standing gap in the textbook market. It is a stimulating and detailed account of the current state of a rapidly moving area of research, aiming to provide the basis for students to be able to follow research papers. This it certainly does.

All of the six sections are heavily biased towards *Drosophila*, but this does not prevent the author meeting his objectives. The section on genome organization covers details of sequence organization and chromatin structure, polytene chromosomes and various models for regulating gene expression. Included under "Mutations" are definitions of what is meant by dominance, dosage compensation and complementation, analysis of complex loci and discussion of how the study of mutations enabled the structure of genes to be unravelled. Hormonally controlled and developmentally regulated genes are described and, finally, mutations which cause lethality at various developmental stages are analysed. In a section on tissue-determination and regeneration the

author concentrates on the production of mosaics and chimaeras to analyse these problems. A special account is devoted to the very topical area of determination mutants where the homoeotic mutants are investigated along with the problems of sex determination; this is particularly useful as many of the molecular studies on these systems demand an understanding of large amounts of background genetics which are rarely documented in such a complete form that students can see how progress has been possible and begin to tackle reading the research papers. The final section on future developments concentrates on cellular systems, a personal view of which areas of research are likely to be fruitful, and the book ends with an analysis of problems still to be solved.

In this book Sang has succeeded in bringing together the classical genetic approach of using mutations to study development with the more recent molecular techniques. I'm sure it will be of great use to students of both genetics and developmental biology. □

Mary Bownes is a Lecturer in the Department of Molecular Biology at the University of Edinburgh.

Nervous connections

Julian Lewis

Development of Nerve Cells and Their Connections.
By W.G. Hopkins and M.C. Brown.
Cambridge University Press: 1984.
Pp.137. Hbk £16, $29.95; pbk £6.95, $13.95.

Principles of Neural Development.
By Dale Purves and Jeff W. Lichtman.
Sinauer/Blackwell Scientific: 1985.
Pp.433. $32.50, £29.50.

From Neuron to Brain, 2nd Edn.
By Stephen W. Kuffler, John G. Nicholls and A. Robert Martin.
Sinauer/Blackwell Scientific: 1984.
Pp.651. Hbk $30; pbk £14.80.

THE development of the nervous system is not yet one of those subjects where a set of fundamental principles emerges neatly from a series of crucial experiments. Perhaps it will be one day: therein lies the challenge. To optimists, the published literature seems like the jumbled pieces of a giant jigsaw puzzle: find the right arrangement, and a beautifully coherent picture will emerge. Others, with less faith in universal principles, may perceive only a rambling mass of natural history anecdotes, fascinating to relate, but with no more logical unity than the Arabian Nights entertainments.

In *Development of Nerve Cells and Their Connections*, Hopkins and Brown adopt a sober and businesslike approach: their short, well-referenced and very reasonably-priced book offers a terse, dispassionate review of what is known about neural development, beginning with the genesis of neurones in the embryo and ending with plasticity and regeneration in the adult. They do not attempt to dramatize the data, and the reader is often left to supply his own emphases. For this reason, and because the presentation is rather condensed and bare of practical details or pictures of the numerous experimental systems discussed, the book, and especially its first half, may be hard going for newcomers to the subject. I found the second half, dealing with the modification of neural connections, the more valuable. As one might expect from these authors, it includes a particularly lucid discussion of synapse elimination and of nerve sprouting. And it is the only one of the books under review that has the courage to tackle head-on, in a few useful and well-organized pages, the problem of learning and memory; the other two venture only a sideways glance at this central enigma.

Purves and Lichtman's larger and more ambitious book, *Principles of Neural Development*, is a masterpiece: comprehensive but not stodgy, clear, accurate, elegantly written, beautifully and copiously illustrated. Even if you don't like words, you will be seduced by the pictures. While it does not offer any revolutionary new synthesis, the book succeeds, better than any previous text on the subject, in the very difficult task of weaving the inchoate mass of data on neural development into a single coherent narrative. Despite the 47 pages of references, and a scholarly care in assigning credit and priorities, Purves and Lichtman avoid the dismal blight that often afflicts *Annual Reviews*, where so many experiments are squeezed into so small a space that no life is left in them. Key experiments are made vivid and concrete by means of figures displaying the methods, materials and observations, reproduced as far as possible from the original papers.

The historical antecedents of modern work are particularly well portrayed. I enjoyed especially the short (one page) biographies of scientists, past and present, who have played major parts in the story. These sketches provide more than the usual bland catalogue of dates and discoveries, managing, in a few paragraphs, not only to explain how and why the scientific achievements were important, but also to bring the characters to life. Purves and Lichtman also have an excellent way with unresolved scientific conflicts: they set out the arguments fairly and squarely, and then tactfully and undogmatically offer their own judgement. As from all the best books, one learns about a great deal more than the title promises, from the principles of Nomarski interference contrast to the discomforts of wearing a reversing pseudophone.

From Neuron to Brain, by Kuffler, Nicholls and Martin, is the second edition of a classic. The first edition was for me and many others a memorable and engrossing introduction to the cellular basis of neurophysiology. It succeeded in large measure by sticking to the excellent principle that it is better — and more fun — to learn ten things well than a hundred things superficially. The authors focused on topics that they knew intimately, and conveyed their fascination with them.

The new edition retains the structure and much of the text of the old, but is enlarged by about 30%. It opens, as before, with a discussion of how neurones represent information about the visual world, updated where appropriate but as good as ever. Spurred on and sustained by the sense that the workings of neurones are the key to the higher functions of the brain, the reader is then taken through the more arduous analysis of the electrical properties of neurones and of synaptic transmission.



Konrad Lorenz with his geese. The picture is reproduced from one of the short biographies in Principles of Neural Development.

The revision here has been more drastic, making the account more rigorous, more complete and less readable for beginners. New topics such as patch clamp recording of currents through single channels are covered clearly and concisely, though sometimes without as much adjustment of the balance of emphasis as they deserve. One does not get the sense that any of the new techniques and discoveries of the past eight years have yielded any radical changes in our perception of neurones or the nervous system, or made any significant part of the material in the first edition obsolete. Is it really true, as stated in the "Concluding Remarks", that "we still do not understand the basic mechanisms that underlie changes in membrane permeability and active transport"?

Subsequent chapters include, together with the old material, many good new things, from sensitization in *Aplysia* to the role of the basal lamina in synapse formation, and a whole new chapter on the control of movement. This is still an outstanding book. □

Julian Lewis is a Lecturer in the Department of Anatomy at King's College, University of London.

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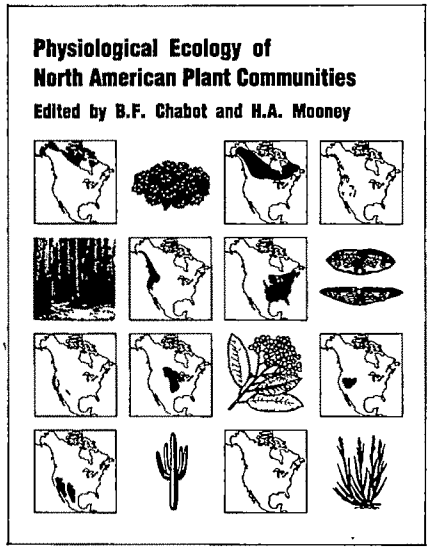
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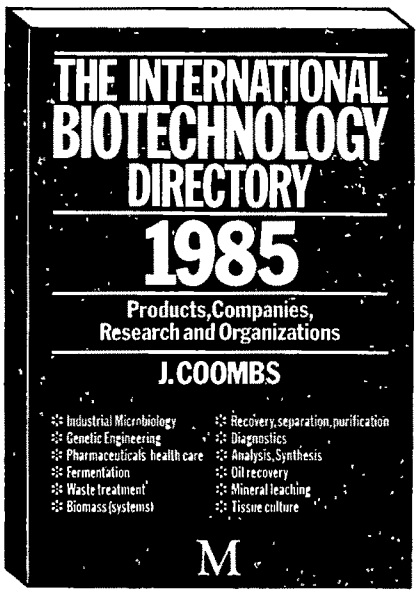
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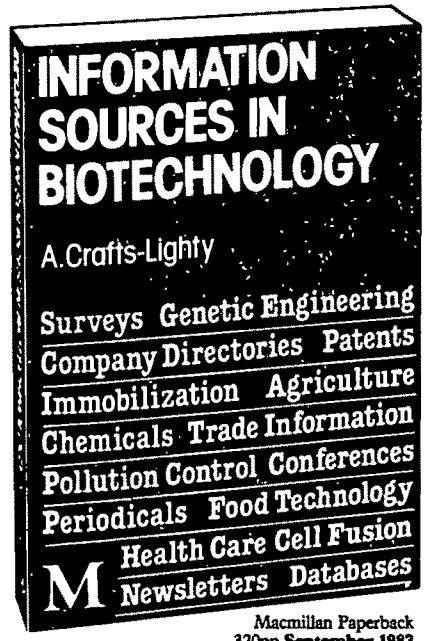
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Ten Feizi

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The hope that hybridoma antibodies would reveal unique cell surface antigens during embryogenesis, differentiation and oncogenesis has been replaced by the realization that such antigens are mainly carbohydrate structures of glycoproteins and glycolipids occurring in many cell types. These findings either may reflect limitations in the methods of selection of hybridoma antibodies or may point to important roles for the diverse carbohydrate structures as receptors for regulators of cell growth and differentiation.

MONOCLONAL antibodies of desired specificity can be produced by the hybridoma technique[1], providing a rapid means of detecting antigens which change during cellular differentiation and maturation (differentiation antigens)[2]. The feasibility of this approach was apparent when a hybridoma antibody of this type was produced incidentally, following immunization of a rat with mouse splenocytes[3]. Serological studies showed that this antibody (M1:22:25:8) which recognizes an antigen with stage-specific expression in the early mouse embryo, becoming restricted only to certain cells in the adult mouse, has Forssman-type specificity[3]. Subsequent biochemical studies[4] confirmed that the antibody recognizes the carbohydrate sequence of the Forssman antigen (structure 20, Table 2), found commonly on glycolipids[5] and occurring also on glycoproteins[6].

Developmental biologists were among the first to use hybridomas to generate 'tailor-made' antibodies to surface antigens of embryonic cells[7]. The antigens, however, seemed elusive; they could not be identified readily by the usual techniques of immune precipitation and polyacrylamide gel electrophoresis. Several of them, including some oncofetal (tumour-associated) antigens, have been shown subsequently to be either carbohydrate sequences on high relative molecular mass (M_r) glycoproteins (or polysaccharides) which cannot be radioiodinated or metabolically labelled with ^{35}S-methionine to sufficiently high specific activities and do not penetrate readily the polyacrylamide gels[8], or they are associated with glycolipids[9]. I review here antigens that have been singled out by monoclonal antibodies as differentiation or tumour-associated antigens and have been shown later to be specific carbohydrate sequences. I discuss their possible roles and conclude by citing some examples of carbohydrate sequences to which biological roles have been assigned without immunological techniques.

Natural monoclonal antibodies

Developmentally regulated antigens were first identified as carbohydrate structures by experiments with certain natural monoclonal antibodies, anti-I and anti-i, occurring in the sera of patients with an autoimmune haemolytic disorder (reviewed in ref. 10) and recognizing two developmentally regulated antigens, I and i, of human erythrocytes. The i antigen is a prominent erythrocyte antigen of the human fetus, diminishing after birth and during the first year of life, while the related antigen, I, increases to the adult concentration[11]. These antigens are now known to be carbohydrate structures on glycoproteins and glycolipids of erythrocytes and other cell types[12-14], consisting of repeating units of *N*-acetyllactosamine (Galβ1→4GlcNAc). This disaccharide sequence is also termed the Type 2 blood group chain[15] or backbone structure[16], to distinguish it from the isomer Galβ1→3GlcNAc (Type 1); both sequences may serve as precursor chains, converted into blood group antigens by further glycosylations. Unbranched oligosaccharides of three or four repeating *N*-acetyllactosamine units joined to each other by a β1→3 linkage (see structure 1, Table 1) express the i antigen[17,18], whereas the corresponding branched structures, formed by β1→6 linkage of *N*-acetyllactosamine units to internal galactose residues of the repeating sequence (as in structure 2, Table 1), express the I antigen[19,20]. During the first year of life, there may be an increased activity of a glycosyltransferase (branching enzyme)[21,101] which transfers *N*-acetylglucosamine via a 1→6 linkage to a previously formed linear chain.

Because undifferentiated cells of the early mouse embryo contain surface glycoproteins with poly-*N*-acetyllactosamine sequences[22], we used monoclonal anti-I and anti-i antibodies against different epitopes on the branched and linear chains to search for such structures both in cells of the early mouse embryo and in cultured embryonal carcinoma cells throughout differentiation and observed that there is a stage-specific expression of the antigens associated with the branched and the linear structures[23,24]. The 'branched' antigen was found on mouse embryos from the zygote stage onwards and the 'linear' antigen became detectable at a later stage (5 days after fertilization) in the first differentiated cells, that is, those of the primitive endoderm. This order of appearance of the branched and linear structures contrasts with that in the human erythrocyte membranes, but the branched structures in the early mouse embryo may be formed by the glycosyltransferases of maternal origin in the oocyte; the linear structures, appearing at onset of differentiation, may represent the first fetal products in this series.

Hybridoma-defined carbohydrate antigens

Following observations on the changing expression of the I and i antigens in the early mouse embryo, it was thought (P. W. Andrews and T. F., unpublished) that the hybridoma antibody anti-SSEA-1 against an antigen appearing at the 8-cell-stage of the mouse embryo[7] might be directed against a carbohydrate structure related to the Ii antigen system. We therefore examined the reactivity of anti-SSEA-1 with a variety of glycoproteins known to express the I and i antigens; we found that anti-SSEA-1 reacts with the intestinal glycoproteins (meconium) of the human fetus[25] and used oligosaccharides to inhibit the binding of the anti-embryo antibody, measured by radioimmunoassay. We established[25,26] that anti-SSEA-1 recognizes α1→3 fucosylated *N*-acetyllactosamine (3-fucosyl-*N*-acetyllactosamine; structure 4, Table 1) and deduced that during mouse development, backbone structures of I-type can be converted into SSEA-1 active structures by α1→3 fucosylation[25]. The 3-fucosyl-*N*-acetyllactosamine sequence is not unique to the mouse embryo; it was first discovered in human ovarian cyst glycoproteins[27], later found among glycolipids of human adenocarcinomas[28] and also in serum glycoproteins and oligosaccharides of human milk

and urine (reviewed in refs 9 and 29). In contrast, this structure in adult mouse occurs only at restricted sites[7]. A second fucose residue joined by $\alpha 1 \rightarrow 2$ linkage to the galactose residue (see structure 6, Table 1) masked completely the expression of the SSEA-1 determinant[25,29], suggesting that the sequential addition (or deletion) of monosaccharides might be one mechanism for the appearance, disappearance and reappearance of antigens during stages of embryogenesis and differentiation[25,30]. The specificity of anti-SSEA-1 was investigated also by structural studies of human erythrocyte glycolipids which contain the SSEA-1 determinant[31-33] and human gastrointestinal glycolipids[34]; there is now agreement that this determinant consists of the 3-fucosyl-*N*-acetyllactosamine structure. An antibody, D_1 56-22, raised against a colon cancer cell line, also recognizes this structure[34]. Furthermore, two or three repeats of this sequence have been detected in some human adenocarcinomas[9].

A variety of monoclonal antibody-defined differentiation antigens of murine and human haematopoeitic or epithelial cells and certain human oncofetal antigens are also carbohydrates, expressed on Type 1 as well as Type 2 backbone sequences or on their additionally glycosylated analogues and may be carried both on glycoproteins and glycolipids (Table 1). Other oncofetal antigens are expressed on carbohydrate chains of the lactosyl-, ganglio- and the globo-series glycolipids (Table 2). Among the structures in Table 1 are those of the major blood group antigens A, B, H, Le^a and Le^b, included first to show their relationship to the antigenic determinants recognized by the anti-mouse embryo antibody anti-SSEA-1 and the anti-human myeloid cell antibodies VEP8 and VEP9, MY-1 and VIM-D5, the anti-human teratocarcinoma antibody FC 10.2, the anti-human adenocarcinoma/adenoma antibodies 19-9, C14, F3 and D_1 56-22, and the anti-milk fat globule antibodies M39 and M18. Second, a number of the monoclonal antibodies MH2, NS-10 and the E1 and the CO series which were thought initially to recognize human adenocarcinoma-associated antigens, were later shown to be directed against the blood group antigens ALe^b/Le^y, B, Le^a or Le^b. Presumably, the tumour cells used as immunogens were derived from individuals with the various blood-group genes.

Carbohydrate antigens have different patterns of expression in various animal species. For example, structure 4 (Table 1) recognized by anti-SSEA-1 is a distinctive antigen of granulocytes among human peripheral blood cells[29,35], but is not expressed on murine, rat, rabbit or porcine granulocytes[36]. Moreover, a carbohydrate antigen may be a normal component in one cell type but a tumour-associated antigen in another. Structure 3 (Table 1), recognized by the monoclonal anti-I antibody Ma, is one such example; this antigen is a normal component of human erythrocytes but is a tumour-associated antigen in gastric adenocarcinomas of individuals who secrete the blood group ABH antigens[37,38]. The biochemical basis of the latter observation may be as follows: the I(Ma) determinant, consisting of the branchpoint sequence $Gal\beta 1 \rightarrow 4GlcNAc\beta 1 \rightarrow 6$ (see Table 1), occurs abundantly among the oligosaccharides of gastric mucosal glycoproteins and secreted gastric mucins[37,38].

Table 1 Differentiation antigens first detected by monoclonal antibodies and identified subsequently as carbohydrate structures based on Type 1 or Type 2 blood group chains

	Structure	Designation of antigen	Cell association
1	$Gal\beta 1 \rightarrow 4GlcNAc\beta 1 \rightarrow 3(Gal\beta 1 \rightarrow 4GlcNAc\beta 1 \rightarrow 3) \geq 2$	i	Fetal erythrocytes, human[17,18], primary endoderm, mouse[23]
2	$Gal\beta 1 \rightarrow 4GlcNAc\beta 1 \rightarrow {}^{6}_{3}Gal\beta 1 \rightarrow 4GlcNAc\beta 1$	I	Adult erythrocytes, human[19]; preimplantation embryos, mouse[23,24]
3	$Gal\beta 1 \rightarrow 4GlcNAc\beta 1 \rightarrow {}^{6}Gal/GalNAc$	I(Ma)	Adult erythrocytes, human[81]; gastric mucosa, non-secretors; gastric adenocarcinomas, secretors[37,38,40]
		M18, M39	Breast epithelium, human[82]
4	$Gal\beta 1 \rightarrow 4GlcNAc$ (↑1,3 $Fuc\alpha$)	SSEA-1 VEP8 and VEP9, Myl, VIM-D5 etc. $D_1$56-22 (X hapten, Le^x)	8-Cell stage mouse embryo[25,26,33] Myeloid cells, human[29,35,83] Colorectal cancer, human[34]
5	$Gal\beta 1 \rightarrow 4GlcNAc$ (↑1,2 $Fuc\alpha$)	TRA-1-85 (blood group H)	Human cell lines*
6	$Gal\beta 1 \rightarrow 4GlcNAc$ (↑1,2 $Fuc\alpha$; ↑1,3 $Fuc\alpha$)	C14 F3 AH6 (Y hapten, Le^y)	Colonic adenocarcinoma, human[84] Lung adenocarcinoma, human[85] Gastric cancer, human[86] Embryonal carcinoma cells, human and mouse[87]
7	$GalNAc\alpha 1 \rightarrow 3Gal\beta 1 \rightarrow 4/3GlcNAc$ (↑1,2 $Fuc\alpha$)	TL5 (blood group A)	EGF receptor of A431 cells[55]
8	$Gal\alpha 1 \rightarrow 3Gal\beta 1 \rightarrow 4GlcNAc$ (↑1,2 $Fuc\alpha$)	E_1 series (blood group B)	Pancreatic cancer[34]
9	$Gal\beta 1 \rightarrow 3GlcNAc\beta 1 \rightarrow 3Gal\beta 1 \rightarrow 4Glc/GlcNAc$	FC10.2	Embryonal carcinoma cells, human[88]; gastric mucosa, non-secretors; gastric adenocarcinoma, secretors[40]
10	$Gal\beta 1 \rightarrow 3GlcNAc$ (↑1,4 $Fuc\alpha$)	CO-514 (blood group Le^a)	Adenocarcinomas, human[89]
11	$Gal\beta 1 \rightarrow 3GlcNAc$ (↑1,2 $Fuc\alpha$; ↑1,4 $Fuc\alpha$)	NS-10 CO-43 (blood group Le^b)	Adenocarcinomas, human[89]
12	$GalNAc\alpha 1 \rightarrow 3Gal\beta 1 \rightarrow 3/4GlcNAc$ (↑1,2 $Fuc\alpha$; ↑1,3/4 $Fuc\alpha$)	G49 MH2 (blood group ALe^b/Le^y)	EGF receptor of A431 cells[90] Colonic adenocarcinoma, human (cited in ref 53)
13	$Gal\beta 1 \rightarrow 3GlcNAc$ (↑2,3 $NeuAc\alpha$; ↑1,4 $Fuc\alpha$)	19 9 (sialyl Le^a)	Colon cancer, human[39]; normal pancreatic ducts[34], normal gastric mucins, non-secretors; gastric cancer mucins, secretors[40], human seminal fluid proteins[41]

* J. K. Picard, P. Goodfellow, P. W. Andrews, G. L. Daniels, K. Uemura & T. Feizi, unpublished observations.

In the majority of the 'secretor' population (whose glycoprotein secretions express the blood group A, B or H antigens of their erythrocytes) the antigenicity of this structure is normally masked by the blood group A, B or H monosaccharides. On the other hand, this antigen is expressed strongly in the mucosal glycoproteins of 25% of the 'non-secretor' population[37]; in these individuals, the I(Ma) determinant presumably is not further glycosylated by the blood group H, A or B monosaccharides and remains accessible. When adenocarcinomas arise in the gastric mucosae of 'secretors', the I(Ma) determinant behaves as a tumour-associated antigen, presumably because the blood group chains are synthesized incompletely.

The sialylated blood group Le^a, (structure 13, Table 1) defined by the hybridoma antibody 19.9 is another tumour-associated antigen found in certain tissues of certain individuals, but a normal antigen in others. This antigen is expressed strongly on glycolipids and glycoproteins extracted from adenocarcinomas of the colon, but not on those of the normal colon[34,39,40], but it has been detected among normal pancreatic glycolipids[34] and normal seminal fluid glycoproteins[41]. In glycoproteins extracted from gastric mucosae, this antigen (like the I(Ma) and FC10.2 antigens), however, is a normal component in 'non-secretors' and a tumour-associated antigen in 'secretors'[40]. The enzymatic basis of these antigenic changes requires investigation, particularly at the level of glycosyltransferase genes.

Apart from carbohydrate determinants shared commonly by glycoproteins and glycolipids, some structures which occur predominantly on glycolipids of the lactosyl, ganglio and the globo series (Table 2), have been shown also to behave as oncodevelopmental antigens recognized by hybridoma antibodies. These include: (1) the *N*-acetylneuraminic acid form of G_{D3} (structure 15, Table 2), recognized by the hybridoma antibodies R_{24} and 4.2 raised against human melanoma cell lines; (2) the 9-*O*-acetyl-*N*-acetylneuraminic acid form of G_{D3}, (structure 16, Table 2), recognized by antibody D1.1 raised against a neuroectodermal antigen of fetal rat and expressed also on human melanoma cells; (3) the blood group P^k determinant, (structure 19, Table 2), recognized by hybridoma 38.13 raised against Burkitt lymphoma cell lines; and (4) two epitopes on structure 21 (Table 2) behaving as the stage-specific embryonic antigens SSEA-3 and SSEA-4 of mouse embryos.

Thus, numerous studies have assigned to carbohydrate structures roles as differentiation antigens and oncofetal antigens. These structures are not confined to any one normal or neoplastic cell type. All such antigens detected by monoclonal antibodies and characterized at the determinant level have been associated with saccharides. These new observations with monoclonal antibodies agree with current knowledge about the genetics and structures of the blood group antigens and their precursors[15,42,43] and the differences in the carbohydrate moieties of the glycolipids of normal and neoplastic cells[9,44] and of various types of differentiated cells[45]. These differences presumably are the end results of either differences in the activities of glycosyltransferases, or in the availability of their substrates, or differences in glycosidase activities in various cell types. Thus, the hope that unique antigenic markers of tumour cells would be discovered and revolutionize tumour diagnosis and therapy is being replaced by the realization that such antigens may not exist.

The failure to discover unique antigenic markers of developmental stage or neoplastic state may reflect limitations in the methods of selection of hybridoma antibodies. This is not necessarily so, for certain autoantibodies and monoclonal antitumour antibodies produced by lymphocyte cell lines derived from patients with neuroectodermal tumours or melanomas are directed also at carbohydrates (structures 17 and 18, Table 2). Thus, the possibility must be considered seriously that: (1) saccharides are the much-sought surface markers that distinguish immature from mature cells and tumour cells from their normal counterparts; (2) the carbohydrate sequences, and/or some additional structures associated with them, may have specific roles in normal cell growth and differentiation; (3) the inappropriate biosynthesis or processing of carbohydrate structures may contribute to the disordered behaviour of tumour cells.

Role of saccharide antigens

Most of these carbohydrate antigens have unknown function. Interestingly, a high proportion of the hybridoma-defined anti-

Table 2 Differentiation antigens first detected by monoclonal antibodies or autoantibodies and identified subsequently as carbohydrate structures of glycolipids of the lactosyl, ganglio or globo series

	Structure	Designation	Cell association
Lactosyl series			
14	Galβ1→4Glc-Cer	T_5A_7 (lactosylceramide)	Myeloid cells, human[91]
15	Galβ1→4Glc-Cer ↑2,3 NeuAcα ↑2,8 NeuAcα	R_{24} 4.2 (G_{D3})	Melanoma, human[92,93]
16	Galβ1→4Glc-Cer ↑2,3 NeuAcα ↑2,8 Neu5,9Ac$_2\alpha$	D1.1	Neuroectoderm, rat, melanoma, human[94]
Ganglio series			
17	GalNAcβ1→4Galβ1→4Glc-Cer ↑2,3 NeuAcα	OFA-1 (autoantigen) (G_{M2})	Melanoma and fetal brain, human[95]
18	GalNAcβ1→4Galβ1→4Glc-Cer ↑2,3 NeuAcα ↑2,8 NeuAcα	OFA-2 (autoantigen) (G_{D2})	Melanoma, human[96] Neuroectoderm, human[97]
Globo series			
19	Galα1→4Galβ1→4Glc-Cer	38.13 (blood group P^k)	Burkitt lymphoma cell lines[98]
20	GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer	M1·22 25·8 (Forssman)	Embryos and embryonal carcinoma cells, mouse[3,4]
21	SSEA-4 NeuAcα2→3Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer SSEA-3	SSEA-3, SSEA-4	4- To 8-cell-stage embryos, mouse[99,100]

gens thus far elucidated are related to the blood group antigen system, possibly reflecting the ease of 'recharacterization' of known structures. Even the amounts of the major blood group antigens A, B and H, in organs of the human fetus change in successive stages of development[46]; this suggests that these carbohydrate structures also are differentiation antigens, but their physiological roles are still unknown. Among some other unexpected developments is the demonstration that most monoclonal antibodies against the receptor for epidermal growth factor (EGF) on the epidermoid carcinoma cell line A431 show specificity for blood group-related carbohydrate structures (reviewed in refs 47, 48, 102). Blood-group and related antigens are expressed strongly on the surface of the epidermoid carcinoma cell line A431 (refs 47, 49); the EGF receptor seems to be the major blood group active glycoprotein in this cell line. One of the monoclonal antibodies (TL5) raised against the EGF receptor of A431 cells[50] recognizes the blood group A antigen[49] and may behave as a growth factor agonist in certain conditions. This antibody does not compete with EGF nor does it have a direct stimulatory effect on fibroblasts, but in an experiment where the cell-bound antibody was crosslinked in the presence of anti-mouse antibodies, the fibroblasts took up tritiated thymidine[50]. If this stimulatory effect is mediated by perturbation of blood group A-active carbohydrate chains, blood group structures are likely to be receptors for certain (as yet undefined) regulators of cell proliferation[48], and systematic studies on the roles of this family of saccharides in the control of cell growth would be encouraged.

One approach to elucidate the roles of the developmentally-regulated carbohydrate structures (such as blood group-related carbohydrate chains based on Type 1 and Type 2 backbones) is to identify the cellular glycoproteins that carry them. This provided the first evidence[51] for their occurrence on the anion transport protein (band 3) of erythrocyte membranes and on high-M_r glycoproteins, including the leukocyte common antigen of human B lymphocytes and the sialoglycoproteins of human T lymphocytes[52,53], just as for the EGF receptor glycoprotein[47]. Well-characterized monoclonal antibodies may be used increasingly for studies of the carbohydrate moieties of glycoproteins and glycolipids.

Another approach is to perturb cell interactions during early embryogenesis (for example, those leading to the process of compaction) by using structurally-defined oligosaccharides as inhibitors. A reversal of compaction of mouse embryos with oligosaccharides expressing the SSEA-1 determinant has been reported[54]. In another study this was not found with free oligosaccharides[55], but an inhibition of compaction in the presence of a trivalent conjugate of this carbohydrate sequence was found[55]. An alternative approach, treating embryos with endo-β-galactosidase, provides evidence for the involvement of carbohydrate chains of poly-*N*-acetyllactosamine type in the compaction process[56]. Compacting embryos (at the 8- to 16-cell stage) decompacted experimentally in calcium-free medium and treated with endo-β-galactosidase, have a dramatically prolonged recompaction time. Endo-β-galactosidase cleaves specifically long-chain oligosaccharide backbones containing the sequence GlcNAc(β1→3Galβ1→3/4GlcNAc)$_n$, abundant on embryonic cells[22]; it abolishes the SSEA-1 activity associated with them[56].

A role for a specific carbohydrate structure in the metastatic properties of B16 melanoma cells of mouse could be that mutant cell lines with reduced metastasizing ability and an increased cell-to-cell adhesive property contain elevated concentrations of α1→3-fucosyltransferase and an increased surface expression of the SSEA-1 determinant[57]. The purified glycosyltransferase can act as a mediator of the adhesion of human fibroblasts[58]. These observations suggest that adhesion and reduced metastasis are mediated by the interaction of this glycosyltransferase on the surface of B16 mutant cells with acceptor substrates on other cells. These and some other observations on galactosyltransferase activities on the surface of embryonal carcinoma cells[59] support the hypothesis[60] that glycosyltransferases of cell surfaces may mediate cell-to-cell adhesion by interacting with acceptor substrates on neighbouring cells. *In vitro* experiments with immobilized α-mannosidase indicate that glycosidases also may serve as cell adhesion molecules[61].

Biological functions of carbohydrates

Biochemical (rather than immunochemical) experiments have allowed the assignment of roles to specific saccharide structures, all of which are potential antigens. For example, the blood anticoagulant activity of heparin results from its ability to bind with high affinity to the protease inhibitor anti-thrombin. This binding is mediated by a specific pentasaccharide sequence on the heparin molecule[62]. There is evidence also for important biological activities associated with other, non-anticoagulant oligosaccharide sequences in heparin: (1) the promotion of angiogenesis by intact heparin and the inhibition of angiogenesis by a hexasaccharide fragment derived from it[63] and (2) the inhibition of smooth muscle proliferation by an oligosaccharide fragment derived from the heparin of endothelial cells[64]. I suggest that the latter observation raises the important possibility that this heparin oligosaccharide sequence may be a receptor analogue for platelet-derived growth factor (PDGF). Some important functions have also been ascribed to structurally-defined sialoglycolipids (gangliosides). The ganglioside GQ_{1b} enhances greatly neurite outgrowth and cell proliferation in neuroblastoma cell lines[65]. Gangliosides G_{M1} and G_{M3} may modulate the phosphorylation (and hence the function) of the receptor for PDGF in a mouse fibroblast (3T3) cell line[66]. These are strong indicators of the importance of carbohydrate structures in the regulation of cell growth.

The role of terminal galactose residues of asialoglycoproteins as recognition structures for their clearance from serum by uptake into hepatocytes[67] and the importance of phosphorylation of mannose residues of lysosomal enzymes for their correct routing to lysosomes[68] are well established. This latter observation indicates clearly that modification of oligosaccharide sequences, for example, by phosphorylation, may change greatly their biological properties.

An important function assigned to specific oligosaccharide sequences in plants has been the eliciting of antimicrobial compounds, phytolexins. There is strong evidence to suggest that the active substances are oligosaccharide fragments released from the injured plant-cell walls and from the cell walls of fungi infecting the plant roots (reviewed in ref. 69). The corresponding carbohydrate binding molecules have not been identified. On the other hand, a variety of carbohydrate binding proteins (lectins) are distributed widely in plant and animal cells[70,103]. Although the precise functions of these lectins, in their tissues of origin are unknown, the biological effects of plant lectins on animal cells, for example, the induction of blast transformation of lymphocytes by phytohaemagglutinin, have been studied widely[70]. In transformed and mitogen-stimulated lymphocytes, we have detected[104] increased levels of proteins antigenically related to an animal lectin (soluble β-galactoside-binding lectin). Thus, it is possible that the lectin and related proteins are somehow involved in the regulation of cell growth. In fibroblasts and epithelial cells, we (S. R. Carding and T.F., cited in ref. 104) have detected several potential receptors for this lectin in the M_r range 10,000–180,000. It will be important to investigate whether growth factor receptors, such as the EGF receptor which contains carbohydrate chains with β-galactoside termini[47], interact with this and other endogenous lectins within or on the surface of cells.

As well as having roles as recognition structures for endogenous ligands, carbohydrate sequences of cell surfaces serve as receptors for infective agents and their toxins[71–75]. They may be important determinants of the tropisms of diverse infective agents. Furthermore, various strains of influenza virus have binding specificities for different sialic acid→galactose linkages and extracellular (soluble) sialoglycoproteins of the host may exert selective pressures resulting in the emergence of variant viruses with altered cell-binding specificities[76]. Thus, the

well-known properties of serum glycoproteins in inhibiting the attachment of viruses to cells and the differing sialyloligosaccharide sequences on the glycoproteins of different animal species may be important in the ecology of the influenza virus.

Studies on the adhesive specificity of the human pathogen *Mycoplasma pneumoniae* indicate that the autoimmune manifestation following infection (the production of high-titre autoantibodies to the erythrocyte antigen I) has its origin in the interaction of the infective agent with saccharide receptors of the host cells, conclusions reached following the observation that the receptors for this agent on human erythrocyte membranes are sialyloligosaccharides of Ii antigen type[77]. Thus, the post-infective anti-I autoantibodies can be considered 'anti-receptor' antibodies directed against the backbone structures of the receptor sequence, raising the possibility that the formation of complexes between the adhesive molecules of infective agents and specific saccharides of host-cell membranes may be an important mechanism for eliciting autoantibodies. The same principle may apply to other autoimmune disorders with anti-receptor antibodies where the causative agents are not yet known but infective agents are not excluded. In those instances where host-cell receptors are saccharide structures shared by several glycoproteins and glycolipids, as with *M. pneumoniae*, there will be cell-type and species differences in the receptor macromolecules[78], reflecting the differences in glycosylations. Hence, the biological effects of a given infective agent will differ accordingly. Presumably, such cell-type differences account for the different effects of *M. pneumoniae* on human T and B lymphocytes; *M. pneumoniae* triggers a mitogenic response in T lymphocytes but induces immunoglobulin secretion without mitogenesis in human B lymphocytes[79]. I suggest that many mechanisms of tropism and some of the transformation-related phenomena of oncogenic viruses are a function of their carbohydrate-binding (lectin-like) proteins.

Conclusions

Monoclonal antibodies have not revealed the much-sought, unique antigen markers of embryonic stage and neoplastic state; few of them may survive the critical assessment of the oncologist for the purpose of tumour diagnosis and therapy[80]. The antibodies characterized already and those awaiting characterization, however, are reagents which will become fundamental analytical tools for future studies of the glycoconjugates of whole cells and their receptors. Although much progress is being made on the structural analysis of glycoconjugates by physicochemical procedures, these are unlikely to supersede the sensitivity of monoclonal antibodies for visualizing the *in situ* disposition of the diverse oligosaccharide sequences in individual cells.

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1 Kohler, G & Milstein, C *Nature* **256**, 495-497 (1975)
2 Milstein, C & Lennox, E *Curr Topics dev Biol* **14**, 1-32 (1980)
3 Willison, K R. & Stern, P L *Cell* **14**, 785-793 (1978)
4 Willison, K R, Karol, R A, Suzuki, A, Kundu, S K & Marcus, D M *J Immun* **129**, 603-609 (1982)
5 Sidiqui, B & Hakomori, S *J biol Chem* **246**, 5766-5769 (1971)
6 Slomiany, B L, Banas-Gruszka, Z, Zdebska, E & Slomiany, A *J biol Chem* **257**, 9561-9565 (1982)
7 Solter, D & Knowles, B B *Proc natn Acad Sci USA* **75**, 5565-5569 (1978)
8 Feizi, T *Biochem Soc. Trans* **11**, 263-265 (1983)
9 Hakomori, S *A Rev Immun* **2**, 103-126 (1984)
10. Feizi, T & Hadler, N in *Biochemical Aspects of Human Disease* (eds Elkeles, R S & Tavill, A S) 656-692 (Blackwell Scientific, Oxford, 1983)
11 Marsh, W L *Br J Haemat* **7**, 200-209 (1961)
12 Feizi, T *Immun Commun* **10**, 127-156 (1981)
13 Hakomori, S *Semin Haemat* **18**, 39-62 (1981)
14 Uemura, K, Childs, R A, Hanfland, P & Feizi, T *Biosci Rep* **3**, 577-588 (1983)
15 Watkins, W M *Adv hum. Genet* **10**, 1-136 & 379-385 (1980)
16 Hounsell, E F & Feizi, T *Med Biol* **60**, 227-236 (1982)
17 Niemann, H, Watanabe, K, Hakomori, S, Childs, R A & Feizi, T *Biochem biophys Res Commun* **81**, 1286-1293 (1978)
18 Gooi, H C *et al Molec. Immun* **21**, 1099-1104 (1984)
19 Watanabe, K, Hakomori, S, Childs, R A & Feizi, T *J biol Chem* **254**, 3221-3228 (1979)
20 Feizi, T, Childs, R A, Watanabe, K & Hakomori, S *J exp Med* **149**, 975-980 (1979)
21 Koscielak, J in *Human Blood Groups* (eds Mohn, J F, Plunkett, R W, Cunningham, R K & Lambert, R M) 143-149 (Karger, Basel, 1977)
22 Muramatsu, T *et al Proc natn Acad. Sci USA* **75**, 2315-2319 (1978)
23 Kapadia, A, Feizi, T & Evans, M J *Expl Cell Res* **131**, 185-195 (1981)
24 Feizi, T, Kapadia, A, Gooi, H C & Evans, M J in *Teratocarcinoma and Embryonic Cell Interactions* (eds Muramatsu, T, Gachelin, G, Moscona, A A & Ikawa, Y) 201-215 (Academic, Tokyo, 1982)
25 Gooi, H C *et al Nature* **292**, 156-158 (1981)
26 Hounsell, E F, Gooi, H C & Feizi, T *FEBS Lett* **131**, 279-282 (1981)
27 Lloyd, K O, Kabat, E A & Licerio, E *Biochemistry* **7**, 2976-2990 (1968).
28 Hakomori, S & Kobata, A in *The Antigens II* (ed Sela, M) 79-140 (Academic, New York 1974)
29 Gooi, H C *et al Eur J Immun* **13**, 306-312 (1983)
30 Feizi, T *Trends biochem Sci* **6**, 333-335 (1981)
31 Nudelman, E *et al Biochem biophys Res Commun* **97**, 443-451 (1980)
32 Hakomori, S, Nudelman, E, Levery, S, Solter, D & Knowles, B B *Biochem biophys Res Commun* **100**, 1578-1586 (1981)
33 Kannagi, R, Nudelman, E, Levery, S B & Hakomori, S *J biol Chem* **257**, 14865-14874 (1982)
34 Hansson, G C *et al J biol. Chem* **258**, 4091-4097 (1983)
35 Huang, L C, Civin, C K, Magnani, J L, Shaper, J H & Ginsburg, V *Blood* **61**, 1020-1023 (1983)
36 Thorpe, S J & Feizi, T *Biosci Rep* **4**, 673-685 (1984)
37 Picard, J, Waldron-Edward, D & Feizi, T *J clin Lab Immun* **1**, 119-128 (1978)
38 Picard, J K & Feizi, T *Molec Immun* **20**, 1215-1220 (1983)
39 Magnani, J L *et al J biol Chem.* **257**, 14365-14369 (1982)
40 Feizi, T *et al Biochem Soc Trans* **12**, 591-596 (1984)
41 Uhlenbruck, G, Van Meensel-Maene, U, Hanisch, F-G & Dienst, C *Hoppe-Seyler's Z physiol Chem* **365**, 613-617 (1984)
42 Kabat, E A *Am J clin Path* **78**, 281-292 (1982)
43 Hounsell, E F & Feizi, T *Med Biol* **60**, 227-236 (1982)
44 Hakomori, S & Young, W W *Scand J Immun* **7**, 97-117 (1978)
45 Karlsson, K-A in *Biological Membranes* Vol 4 (ed Chapman, D) 1-74 (Academic, London, 1982)
46 Szulman, A E *Curr Topics dev Biol* **14**, 127-145 (1980)
47 Childs, R A *et al EMBO J* **3**, 2227-2233 (1984)
48 Feizi, T & Childs, R A *Trends biochem Sci* **10**, 24-29 (1985)
49 Gooi, H C *et al Biosci. Rep* **3**, 1045-1052 (1983)
50 Schreiber, A B, Libermann, T A, Lax, I, Yarden, Y & Schlessinger, J *J biol Chem* **258**, 846-853 (1983)
51 Childs, R A, Feizi, T, Fukuda, M & Hakomori, S *Biochem J* **173**, 333-336 (1978)
52. Childs, R A & Feizi, T *Biochem Biophys Res Commun* **102**, 1158-1164 (1981)
53 Childs, R A *et al. Biochem biophys Res Commun* **110**, 424-431 (1983)
54 Bird, J M & Kimber, S J *Devl Biol* **104**, 449-460 (1984)
55 Fenderson, B A, Zehavi, U & Hakomori, S *J exp Med* **160**, 1591-1596 (1984)
56 Rastan, S *et al J Embryol. exp Morph* (in the press)
57 Finne, J *Biochem Soc. Trans* **11**, 269-270 (1983)
58 Rauvala, H, Prieels, J-P & Finne, J *Proc natn Acad Sci USA.* **80**, 3991-3995 (1983)
59 Shur, B D *Molec. cell Biochem* **61**, 143-158 (1984)
60 Roseman, S *Chem Phys Lipids* **5**, 270-297 (1970)
61 Rauvala, H & Hakomori, S *J Cell Biol.* **88**, 149-159 (1981)
62 Lindahl, U, Backstrom, G & Thunberg, L *J. biol Chem.* **258**, 9826-9830 (1983)
63 Folkman, J, Langer, R, Linhardt, R J, Haudenschild, C & Taylor, S *Science* **221**, 719-725 (1983)
64 Castellot Jr, J J, Favreau, L, Rosenberg, R D & Karnovsky, M J *J biol Chem* **257**, 11256-11260 (1982)
65 Tsuji, S, Arita, M & Nagai, Y *J Biochem* **94**, 303-306 (1983)
66 Bremer, E G, Hakomori, S, Bowen-Pope, D F, Raines, E & Ross, R *J biol Chem* **259**, 6818-6825 (1984)
67 Ashwell, G & Hartford, J *A Rev Biochem* **51**, 531-554 (1982)
68 Sly, W S & Fischer, H D *J cell Biochem.* **18**, 67-85 (1982)
69 Darvill, A G & Albersheim, P *A Rev Physiol* **35**, 243-275 (1984)
70 Sharon, N *Adv Immun* **34**, 213-298 (1983)
71 Svennerholm, L, Fredman, P, Elwing, H, Holmgren, J & Strannegård, Ø in *Cell Surface Glycolipids* (ed Sweeley, C C) 373-390 (ACS Symp 128) (American Chemical Society, Washington, DC, 1980)
72 Leffler, H & Svanborg Eden, C *FEMS Microbiol Lett* **8**, 127-134 (1980)
73 Markwell, M A K, Svennerholm, L & Paulson, J C *Proc. natn. Acad Sci USA* **78**, 5406-5410 (1981)
74 Hansson, G C *et al FEBS Lett* **170**, 15-18 (1984)
75 Suzuki, T, Harada, M, Suzuki, Y & Matsumoto, M *J Biochem, Tokyo* **95**, 1193-1200 (1984)
76 Rogers, G N, Pritchett, T J, Lane, J L & Paulson, J C *Virology* **131**, 394-408 (1983)
77 Loomes, L M *et al Nature* **307**, 560-563 (1984)
78 Feizi, T *et al Biosci Rep* **4**, 743-749 (1984)
79 Biberfield, G *Scand J Immun* **6**, 1145-1150 (1977)
80 Feizi, T *Biochem Soc. Trans* **12**, 545-549 (1984)
81 Feizi, T, Kabat, E A, Vicari, G, Anderson, B & Marsh, W L *J Immun* **106**, 1578-1592 (1971)
82 Gooi, H C *et al. Carbohydr Res* **120**, 293-302 (1983)
83. Gooi, H C *et al Clin exp Immun* (in the press)
84 Brown, A *et al Biosci Rep* **3**, 163-170 (1983)
85 Lloyd, K O, Larson, G., Stromberg, N, Thurin, J & Karlsson, K-A *Immunogenet* **17**, 537-541 (1983)
86 Abe, K, McKibbin, J M & Hakomori, S *J biol Chem* **258**, 11793-11797 (1983)
87 Blaineau, C, Le Pendu, J, Arnaud, D., Connan, F & Avner, P *EMBO J* **2**, 2217-2222 (1983)
88 Gooi, H C *et al Molec. Immun* **30**, 607-613 (1983)
89 Blaszczyk, M *et al Archs Biochem Biophys* **233**, 161-168 (1984)
90 Gooi, H C *et al Molec. Immun* (in the press)
91 Symington, F W, Bernstein, I D & Hakomori, S *J biol Chem* **259**, 6008-6012 (1984)
92 Pukel, C S *et al J exp Med* **155**, 1133-1147 (1982)
93 Nudelman, E *et al J biol Chem* **257**, 12752-12756 (1982)
94 Cheresh, D A, Reisfeld, R A & Varki, A P *Science* **225**, 844-846 (1984)
95 Tai, T, Paulson, J. C, Cahan, L D & Irie, R F *Proc. natn Acad Sci. USA* **80**, 5392-5396 (1983)
96 Watanabe, T *et al J exp Med* **156**, 1884-1889 (1982)
97 Cahan, L D, Irie, R F, Singh, R, Cassidenti, A & Paulson, J C *Proc. natn Acad Sci. USA* **79**, 7629-7633 (1982)
98 Nudelman, E *et al Science* **220**, 509-511 (1983)
99 Kannagi, R *et al J biol. Chem* **258**, 8934-8942 (1983)
100 Kannagi, R *et al EMBO J* **2**, 2355-2361 (1983)
101 Piller, F *et al J biol Chem* **259**, 13385-13390 (1984)
102 Gooi, H C *et al Biosci Rep* (in the press)
103 Barondes, S H, *Science* **223**, 1259-1264 (1984)
104 Carding, S R, Thorpe, S J, Thorpe, R & Feizi, T, *Biochem Biophys Res Commun* (in the press)

Importance of the 'shape' of the melting regime during partial melting of the mantle

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The amount of melt formed in individual stream lines of upper mantle rocks passing through a partial melting regime increases from zero at the edge to a maximum at the central axis. Relationships between aggregated liquid compositions from such melting regimes and the source mantle should be obained by integration of the appropriate equations for liquid composition taking into account the horizontal and vertical form of the melting regime. Misinterpretation of integrated liquid products from such regimes would be easy if too simple a model of the melting process is used.

GEOCHEMICAL effects of partial melting of upper mantle rocks have been explored over the past 16 years using relationships developed by Gast[1], Shaw[2] and Langmuir *et al.*[3]. Applications of these relationships to the prediction or interpretation of data sets for volcanic rocks have assumed implicitly that all parcels of the mantle processed through the partial melting regime undergo an equal amount of melt removal. Yet it was known throughout this time that the maximum temperature above the solidus and, therefore, the mass fraction of melt developed in parcels of mantle passing through the melting regime, will be at a maximum along the central axis and will decline to zero at the edges[4].

Consider one example of the consequence of using more sophisticated models—the case where mass fraction (F) of melt developed on each stream line increases to some central maximum (F_m) as a linear function of distance inward from the periphery, in a melting regime of semi-infinite lateral extent. Only half as much liquid will be developed in this first case as would be developed in a second, or 'simple', case where all the mantle which had encountered the melting regime underwent the same (maximum F_m) mass fraction of partial melting.

Now consider a totally incompatible element, wholly concentrated in the first drop of liquid to form along each stream line. The concentration of this element in the liquid derived in the first case will necessarily be just twice its concentration in the liquid derived in the second case. If the melting regime were circular in plan (for example, a plume) rather than of semi-infinite linear extent (for example, a mid-ocean ridge segment) the amount of liquid developed will be one-third that developed in the 'simple' case and the concentration of the totally incompatible element consequently will be three times that in the liquid derived in the 'simple' case.

Most attempts to invert the geochemistry of erupted basalt magmas to predict upper mantle chemistry, or to predict potential basalt compositions which might form by partial melting of particular mantle materials, have used the equations[1-3] which relate liquid, residue and source mantle compositions in the 'simple' case mentioned above. Clearly, when this is done inappropriately, there will be a tendency towards some combination of: (1) underestimation of the mass fraction of melting involved relative to the maximum amount actually developed (F_m); and (2) overestimation of the content of the totally incompatible element in (that is, the 'fertility' of) the source mantle.

Adoption of the more sophisticated melting model allows an immediate reconcilliation between field, petrographic and major element chemical features in upper mantle materials which point to large ($F_m \sim 0.25$-0.35) maximum mass fractions of melt removal (see, for example, ref. 5) and features of trace-element geochemistry which seem to point to very small mass fractions of partial melt removal (see, for example, ref. 6). The incompatible trace-element chemistry of the aggregated melts is dominated by liquid contributions from the periphery of the melting regime, where the mass fraction of melting is indeed small, whereas events in the central region tend to dominate the other aspects mentioned.

Adoption of the more sophisticated model has some relevance to the interpretation of the apparently higher 'fertility' (deduced using 'simple' models) of the mantle sources[7] which feed 'plume' type volcanism. Eventually, this model may reconcile the apparent fertility of plumes with the undeniable fact that significantly fertile mantle is too iron-rich, too well endowed with spinel and garnet and hence too dense to rise through residual mantle[8-11]. In principle, the sophicated model permits a less fertile mantle material, rising in a circular plan-form plume, to yield a liquid more enriched in incompatible elements than is obtained from neighbouring more fertile mantle undergoing melting in a linearly extensive melting regime.

Geochemical features suggestive of mixing of magmas from two or several sources when 'simple' models are used[12,13] may derive at least in part from the fact that in the sophisticated model the primary magmas are indeed complex mixed melts.

It may seem that the trace-element geochemistry of erupted basalts can still be inverted viably to yield upper mantle source characteristics using the 'simple' relationships with some average value for the mass fraction of liquid developed from the whole mass of mantle passing through the melting regime. But this is only true for totally incompatible elements.

Integration of the relationships described below yields expressions which in turn predict the liquid and residue compositions that might be produced in sophisticated melting regimes. These compositions cannot be inverted safely for elements which are not totally incompatible, using any average value of the mass fraction of partial melting inserted into the 'simple' relationships. Highly incompatible element concentrations predicted by the sophisticated model are lower than expectations from the best fit 'simple' model using a single average value of the melt fraction.

Discrimination of concentration between two highly incompatible elements of very similar distribution coefficient is achieved much more efficiently in the melts predicted by the sophisticated model. In 'simple' models, such discrimination can only be produced at very small mass fractions of partial melting. Interpretations based on 'simple' models (which may not be appropriate) may lead towards erroneous conclusions that particular magmas have been derived by exceptionally small mass fractions of partial melting or by extraction from mantle sources which have been depleted of their most highly incompatible elements in a previous small-scale melt extraction event.

Aggregated melt composition relationships

The treatment which follows is simplified greatly; it is used to explore some first-order effects which will result from the adoption of more sophisticated models of melting regimes, and is considered as an intermediate advance rather than a conclusion. (Several points for further refinement are noted in passing.) Partial melting is assumed here to result from the decompression of hot mantle ascending along an adiabat intersecting the solidus curve[4], but this assumption is not critical in the mathematical formulation.

The 'shape' of the melting regime has two components. The plan-form 'shape' is assumed to be either linear or circular (but solutions for the elliptical end-form would be desirable). It is further assumed that the amount of melt extracted is a maximum on the central axis or at the centre and falls off symmetrically towards the periphery. All melt extracted is assumed to be aggregated and tapped at the central axis only (tapping of liquids developed from sub-portions of a large melting regime has obvious relevance to the Galapagos Islands and sea mounts near spreading centres).

The cross-section 'shape' of the melting regime is defined here by the relationship between distance, y, from the edge of the melting regime (Fig. 1) relative to Y_m, the distance to the centre of the melting regime and F_y the maximum mass fraction of melt extracted from a parcel of mantle at distance y from the edge, relative to F_m (the maximum mass fraction of melt extracted from a parcel of mantle passing along the central axis of the regime). Melting of sub-spherical blobs is not addressed here.

The 'parcel' of mantle is defined as a very small mass or volume of mantle of width dy (unit length perpendicular to the cross section) and height dh (such that an additional mass fraction of partial melt, dF_y is produced within it as it moves upwards a distance, dh, along the stream line).

The appropriate relationships have then been integrated[14] on the following assumptions: First,

$$(y/Y_m) = (F_y/F_m)^n \quad (1)$$

where $n > 0$ (alternative assumptions for the relationship might yield a more easily integrated expression, advantageous because it would allow inversion of data sets to obtain the important parameters F_m and n).

Second, the partial melting process affecting each individual parcel of mantle produces a continuous partial melt (CPM), a process in which some mass fraction of the residue is trapped liquid, but in which further liquid is drained continuously from the parcel as melting proceeds. Each new infinitesimal increment of liquid formed is well mixed with the trapped liquid and balanced by the infinitesimal decrement of melt removed from that mantle parcel at the same step. The process has been modelled using the familiar relationship[2] for the composition of a perfect fractional partial melt (PFPM).

$$C_L/C_S = \frac{(1-f_y)^{1/D^*-1}}{D^*} \quad (2)$$

C_L is the concentration of an element in the liquid present in (or removed from) the parcel at the stage when mass fraction f_y of the original parcel has been removed by the CPM process (this parcel of mantle will yield ultimately a maximum mass fraction of melt F_y, where $F_y < F_m$). C_S is the concentration of the element in the original mantle parcel before the onset of melting and melt removal. The effects of trapped liquid are taken into account in the formulation of the bulk distribution coefficient, D^* as

$$D^* = D(1-z)+z \quad (3)$$

where D = concentration of element in the bulk crystal assemblage divided by the concentration of element in the liquid and z is the mass fraction of the trapped liquid residue. (Trapped liquid is treated simply as another residual phase for which the distribution coefficient happens to be unity.)

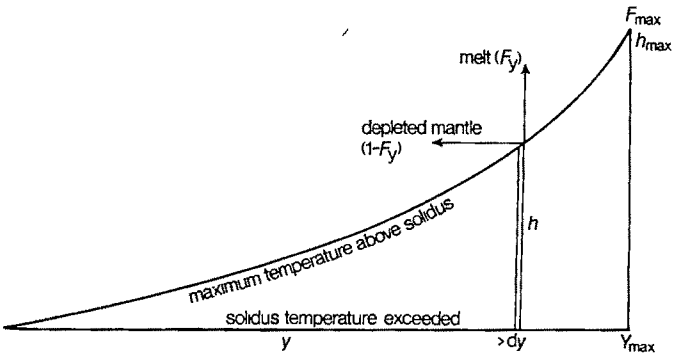


Fig. 1 Half cross-section of a hypothetical upper mantle melting regime (adapted and simplified from ref. 4) illustrating the relationships between the distance y of a stream-line, height h, in the partial melting regime and Y_m, the distance to the centre. The mass fraction F_y of melt extracted along this stream line is assumed proportional to h and the 'shape' of the melting regime is defined by the relationship between y and F_y (see text). Arrows labelled 'depleted mantle' and 'melt' are stylized and indicate no more than physical separation of melt from residue somewhere in the regime.

Equations (2) and (3) yield a precise description of the process at all values of D, z and f_y (full integration from mass balance equation on request from the author) and no problems arise with melt not collected from the periphery because f_y, F_y and F_m are defined as the mass fractions of melt which are collected.

Third, z, D and hence D^* are assumed constant for all parcels of mantle throughout the melting process and C_S is also assumed constant in time and space. (Subsequent developments must examine the consequences of the variations in D which accompany both the changes in the proportions of the crystal species which will occur when F_m is large and the changes in phase assemblage and liquid structure which will occur when the melting regime extends through a significant range of pressure. In principle, these complications can be introduced into the numerical integration of the relationships derived below, but the assumptions made here are necessary for the analytical solutions presented.) Note, however, that for very highly incompatible elements with $D < 0.005$, the value of D^* will be dominated by the mass fraction of trapped liquid, z, if this is greater than ~0.005 (0.5%).

Fourth, all decrements of melt removed from all parcels of mantle are aggregated without any further interaction with source material or residues, and well mixed to form an accumulated perfect fractional partial melt (APFPM) or an accumulated continuous partial melt (ACPM). The composition of the APFPM or ACPM derived from a single mantle parcel during its passage through the melting regime, or from all parcels along a single mantle stream-line through the regime at one instant, is then obtained from the familiar relationship[2]

$$\bar{C}_L/C_S = \{1-(1-F_y)^{1/D^*}\}/F_y \quad (4)$$

which is the integral of equation (2) between $f_y = 0$ and $f_y = F_y$.

Fifth, residue stream-line patterns and F_y along each flow line are assumed to remain constant with time (that is, the process is in a steady state).

Sixth, the APFPMs or ACPMs from each mantle stream line are in turn aggregated to yield a complex accumulated perfect fractional (or continuous) partial melt (CAPFPM or CACPM) which constitutes the primary magma segregated from the partial melting regime. The relationship between the concentration of an element $\bar{C}_L$ in this CAPFPM or CACPM and its concentration in the source C_S, is obtained by integrating $\bar{C}_L/C_S$ (equation 4) for $F_y = 0 \rightarrow F_m$ over the cross-section shape and along (linear) or around (circular) the plan-form of the melting regime, as

follows:

$$\bar{C}_L/C_S = \left\{\int_0^{F_m}\left[\begin{matrix}\text{relative length in planform}\\ \text{to which result applies}\end{matrix}\right]\times\left[\begin{matrix}\text{concentration of element}\\ \text{in liquid increment}\end{matrix}\right]\times\left[\begin{matrix}\text{mass fraction of liquid}\\ \text{having this concentration}\end{matrix}\right]dy\right\} - \left\{\int_0^{F_m}\left[\begin{matrix}\text{relative length in planform}\\ \text{to which result applies}\end{matrix}\right]\times\left[\begin{matrix}\text{mass fraction of liquid}\\ \text{having this concentration}\end{matrix}\right]dy\right\} \quad (5)$$

In the linear planform case the relative lengths in planform are all the same, hence the first bracket top and bottom becomes unity. In the circular planform case the relative lengths are given by $2\pi(1-y/Y_m)$ which is $2\pi(1-(F_y/F_m)^n)$. The middle bracket on the top line contains the expression from equation (4) above. The last bracket, top and bottom, is simply F_y.

Substituting, from equation (1), $dy=(nY_m/F_m^n)F_y^{n-1}\,dF_y$ and cancelling constants n, F_m, Y_m in top and bottom lines, one obtains in the linear planform case

$$\frac{\overset{\text{CAPFPM}}{\underset{\text{LINEAR}}{\bar{C}_L}}}{C_S} = \frac{\int_0^{F_m}\left[\frac{1-(1-F_y)^{1/D^*}}{F_y}\right]F_yF_y^{n-1}\,dF_y}{\int_0^{F_m}F_yF_y^{n-1}\,dF_y} \quad (6)$$

or, in the circular plan case

$$\frac{\overset{\text{CAPFPM}}{\underset{\text{CIRCULAR}}{\bar{C}_L}}}{C_S} = \frac{\int_0^{F_m}\left[1-\left(\frac{F_y}{F_m}\right)^n\right]\left[\frac{1-(1-F_y)^{1/D^*}}{F_y}\right]F_yF_y^{n-1}\,dF_y}{\int_0^{F_m}\left[1-\left(\frac{F_y}{F_m}\right)^n\right]F_yF_y^{n-1}\,dF_y} \quad (7)$$

Both expressions may be further simplified by cancellation of an F_y in the top line and summing powers of F_y in the bottom line before commencing numerical or analytical integration.

Seventh, the derivation of equations (5) and (6) assumes that each decrement of melt removed from each parcel of mantle undergoes no further chemical interaction with any other parcel of mantle or residue, but this represents only one extreme possible assumption. Another (not the only other) extreme assumption is that each decrement of melt flows upward along the mantle stream line, remaining in perfect equilibrium with the residues, and is then aggregated. This leads to the alternative relationship to that given in equation (4),

$$\bar{C}_L/C_S = 1/[D^*+F_y(1-D^*)] \quad (4')$$

which is familiar[2] as a form of the relationship for the composition of a perfect equilibrium partial melt (PEPM) product developed where mass fraction F_y of the original source has become removable melt. That this is true can be demonstrated by integration of the appropriate mass balance equations, but may also be appreciated intuitively. All the melt (mass fraction F_y) from the whole stream line must pass through and equilibrate with the final residue, mass fraction $(1-F_y)$ leading to equation (4') by simple mass balance. (Note the separate increments of melt composing the mass fraction F_y commenced their travel at different times). Equation (4') is valid provided n and F_m are constant, which is not guaranteed in real geological situations.

Integration as before then yields the alternative expressions for the linear plan case

$$\frac{\overset{\text{CAPEPM}}{\underset{\text{LINEAR}}{\bar{C}_L}}}{C_S} = \frac{\int_0^{F_m}\left[\frac{1}{D^*+F_y(1-D^*)}\right]F_yF_y^{n-1}\,dF_y}{\int_0^{F_m}F_yF_y^{n-1}\,dF_y} \quad (6')$$

and for the circular plan case,

$$\frac{\overset{\text{CAPEPM}}{\underset{\text{CIRCULAR}}{\bar{C}_L}}}{C_S} = \frac{\int_0^{F_m}\left[1-\left(\frac{F_y}{F_m}\right)^n\right]\left[\frac{1}{D^*+F_y(1-D^*)}\right]F_yF_y^{n-1}\,dF_y}{\int_0^{F_m}\left[1-\left(\frac{F_y}{F_m}\right)^n\right]F_yF_y^{n-1}\,dF_y} \quad (7')$$

Computation of the expressions in equations (6), (7), (6') and (7') is best carried out by numerical integration, particularly for the case of very highly incompatible elements with low values of $D^*(<0.003)$. Analogous expressions may be written to describe the average residue compositions[14].

However, integration of equations (6) and (7) by substitution by the chain rule and by parts, yields in the general case, an infinite series[14] which may be rearranged in the forms given in equations (8) and (9) below for the linear and circular plan forms respectively.

$$\frac{\overset{\text{CAPFPM}}{\underset{\text{LINEAR}}{\bar{C}_L}}}{C_S} = \left[\frac{n+1}{n}\right]\left\{\left[\frac{1-(1-F_m)^{1/D^*}}{F_m}\right]-\left[\frac{(1-F_m)^{1/D^*-1}}{D^*}\right]\{\text{series 1}\}\right\} \quad (8)$$

where

$$\{\text{series 1}\} = \left\{\frac{1}{(n+1)}+\frac{F_m}{(1-F_m)D^*}\frac{(1-D^*)}{(n+1)(n+2)}+\cdots+\frac{Q^{p-1}n^!(1-D^*)(1-2D^*)\cdots(1-(p-1)D^*)}{(n+p)!}\right\}$$

and where $Q=[F_m/(1-F_m)D^*]$ and the general term is written for the pth term of the subseries, designated here as {series 1}.

$$\frac{\overset{\text{CAPFPM}}{\underset{\text{CIRCULAR}}{\bar{C}_L}}}{C_S} = \left[\frac{(n+1)(2n+1)}{2n^2}\right]\times\left\{\left[\frac{1-(1-F_m)^{1/D^*}}{F_m}\right]-\left[\frac{(1-F_m)^{1/D^*-1}}{D^*}\right]\times[2\{\text{series 1}\}-\{\text{series 2}\}]\right\} \quad (9)$$

where

$$\{\text{series 2}\} = \left\{\frac{1}{2n+1}+\frac{Q(1-D^*)}{(2n+1)(2n+2)}+\cdots+\frac{Q^{p-1}(2n)!(1-D^*)(1-2D^*)\cdots(1-(p-1)D^*)}{(2n+p)^!}\right\}.$$

The term $(1-(p-1)D^*)$ in the general term of these series must eventually become negative for $D^*>0$ and successive terms thereafter are alternately positive and negative. The series will converge provided $F_m/(1-F_m)<1$, that is, $F_m<0.5$ (the majority of geologically reasonable cases) and evaluation will terminate, with all later terms being zero, at the $(1/D^*+1)$th term if $1/D^*$ is an integer.

Equations (8) and (9) may be simplified for analytical purposes to

$$\left[\begin{matrix}\text{Complex}\\ \text{aggregated}\\ \text{melt}\end{matrix}\right] = \left[\begin{matrix}\text{shape}\\ \text{factor}\end{matrix}\right]\left\{\left[\begin{matrix}\text{APFPM}\\ \text{at } F_{max}\end{matrix}\right]-\left[\begin{matrix}\text{PFPM}\\ \text{at } F_{max}\end{matrix}\right]\left[\begin{matrix}\text{series}\\ \text{factor}\end{matrix}\right]\right\} \quad (10)$$

Equation (10) may be stated as follows: The concentration of an element in a CAPFPM or CACPM product will be that expected in an APFPM (or ACPM) product developed at the maximum mass fraction of partial melting minus the concentration of that element developed in the last extracted drop of a PFPM (or CPM) product at the same maximum mass fraction of melting multiplied by a series term whose value is a function of F_m, D^* and the power function n; the whole of the above multiplied by a shape factor which is a function of the power n alone.

Evaluation of the relationships

Some important properties of equations (8) and (9) are: First, when $D^*=0$ (totally incompatible element), $\bar{C}_L/C_S=$[shape

factor][$1/F_m$]. Second, when $D^* = 1$, $\bar{C}_L/C_S = 1$ (logical). Third, when $D^* \to \infty$ (totally compatible element), $\bar{C}_L/C_S \to 0$ (logical). Fourth, when $0 < D^* < 1$, $\bar{C}_L/C_S$ is less than would be expected in a simple APFPM product at the appropriate F_m multiplied by the shape factor. The quantity by which it is less is given by [PFPM at F_m] {series factor} [shape factor]. As D^* becomes smaller, the [PFPM at F_m] term passes through a maximum where $D^* \simeq F$ and thereafter becomes very rapidly smaller. Simultaneously, the {series factor} increases in magnitude. The net effect is that this subtracted quantity passes through a maximum (and $\bar{C}_L/C_S$ through a minimum relative to the simple APFPM term) at some intermediate value of D^* which is a function of F_m and n. The resultant values of $\bar{C}_L/C_S$ nevertheless seem to discriminate increasingly efficiently between elements with very small but fixed differences of D^* as D^* itself decreases.

Fifth, as $n \to 0$ (the mass fraction of partial melting is small over most of the regime but increases sharply close to the central axis), the [shape factor]$\to \infty$ and $\bar{C}_L/C_S$ tends to very large values when $D^* < 1$, very small values when $D^* > 1$. The parameters $n = 1$, $D^* = 0$ describe the case discussed in the introduction. Sixth, when $n \to \infty$ (the mass fraction of melt extracted is very close to F_m for all flow lines through the regime) the differences between equations (8) and (9) disappear since all terms in {series 1} and {series 2} tend to zero. The [shape factor]$\to 1$, and $\bar{C}_L/C_S \to [1-(1-F_m)^{1/D^*}]/F_m$ which in the limit is identical with equation (4).

Seventh, the [APFPM] term in equation (10) yields concentrations which differ little from those of PEPM (see equation 4'), particularly when F_m is substantial. When F_m is substantial (0.2–0.4), this term is very inefficient at discriminating between very highly incompatible elements with small differences of D^*. To obtain good discrimination using this this term, it is necessary to reduce F_m to values ~0.01 rather than ~0.3.

Eighth, the [PFPM] term in equation (10) yields results which differ dramatically from the [APFPM] term when D^* is small and F_m is substantial. Moreover, the [PFPM] term discriminates very efficiently between very highly incompatable elements of very similar D^*, but at the cost of generating minute concentrations of these elements in the [PFPM] product when F_m is substantial. For very highly incompatible elements this term would be insignificant were it not for the multiplier contained in the series term (see below).

Finally, the {series factor} in equation (10) may contain some very large terms, particularly when F_m is large and D^* and n are small. When D^* is small, the largest terms in the series are grouped about $1/2D^*$ terms into the series; all the first $1/D^*$ terms are positive and all other terms are small (for example, if $D^* = 0.001$, the largest terms contributing to the {series factor} are ~500 terms into the series, and involve evaluation of quantities such as $Q^{499} \simeq 400^{499}$; $(n+p)! \sim 501!$).

Geological significance

These and other properties[14] of equations (8), (9) and (10) have important implications for the interpretation of major and trace element geochemistry and experimental petrology data for natural lavas, which include:

(1) Even true primary magmas (developed according to the above scenario) would not be in phase or chemical equilibrium with any specific residue composition (although the discrepancy may be small in some respects); nor would the bulk CAPFPM product be in (conventional) chemical equilibrium with the bulk residue from the process.

(2) ('Effective' bulk distribution coefficients, defined as D' = (concentration of element in CAPFPM bulk residue) divided by (concentration of element in CAPFPM bulk liquid extract), may differ markedly from D or D^*. In the case of very highly incompatible elements when n is small and z relatively large, D' may be much larger than D, thus reducing greatly the efficiency of the partial melting process in 'stripping' the mantle of its very highly incompatible elements.

(3) By virtue of the [shape factor], concentrations of very highly incompatible elements in CAPFPM products will be significantly higher in the linear plan case than in simple APFPM products of the same source mantle and higher still in the circular plan case, even if the power factor n remains constant. By virtue of higher thermal losses per unit volume from a circular plan regime than from a linear plan regime, the power factor n is likely to be smaller in the circular plan case than in the linear plan case, enhancing the effect already noted. These effects could be misinterpreted easily as products of mantle heterogeneity, as outlined in the introduction.

(4) The CAPFPM product is much more efficient at fractionating two very highly incompatible elements of similar D^* than is a 'simple' APFPM product a equivalent F or an 'average' value of F (see ref. 14). This effect could reinforce the readily available misinterpretation (see (3) above) that very small mass fractions of partial melting are required to explain the geochemistry of erupted products; or lead to a further misinterpretation that D^* values must be still lower, that is, that mass fractions of trapped melt in the residual mantle must be lower than is actually the case.

(5) There is a disturbing resemblance between the potential misinterpretations of CAPFM products identified above and the principal conclusions of most publications in the field of igneous geochemistry in the past 15 yr containing interpretations based upon simpler models of the partial melting process. I do not argue here that there are no differences in 'fertility' of different portions of the mantle, or that small mass fractions of partial melt are not, on occasion, extracted, or that large portions of the mantle have not suffered a small fractional melt extraction at some earlier stage of their history. These are probable and readily acceptable conclusions. I do, however, suggest that the geochemical evidence needs to be re-evaluated in order to remove any spurious effects and,thereby, place these conclusions on a more secure foundation.

Alternative assumption integration

Equations (6') and (7') may also be integrated to yield results of the form

$$\begin{bmatrix}\text{Complex}\\ \text{aggregated}\\ \text{melt}\end{bmatrix} = \begin{bmatrix}\text{shape}\\ \text{factor}\end{bmatrix}\begin{bmatrix}\text{PEPM}\\ \text{at } F_{max}\end{bmatrix}\begin{bmatrix}\text{series}\\ \text{factor}\end{bmatrix} \qquad (10')$$

Shape factors are as before; the PEPM term at F_m is analogous to equation (4') above and the {series factor} in equation (10') is either

{series 3}

$$= \left\{\frac{nn!}{(n+1)!} + \frac{Snn!}{(n+2)!} + \frac{2S^2nn!}{(n+3)!} + \cdots + \frac{(p-1)!S^pnn!}{(n+p)!}\right\}$$—linear case

or

$$[2 \times \{\text{series 3}\} - \{\text{series 4}\}]$$—circular case

where

$$\{\text{series 4}\} = \left\{\frac{2n(2n)!}{(2n+1)!} + \frac{S2n(2n)!}{(2n+2)!} + \cdots + \frac{(p-1)!S^p2n(2n)!}{(2n+p)!}\right\}$$

and $S = [F_m(1-D^*)]/[D^* + F_m(1-D^*)]$.

These relationships differ from those given in equations (8) and (9) above in that they do not contain the subtracted term which produces the strong discrimination between elements of like distribution coefficient. Although it is true that the [PEPM] term produces much the same values of C_L as the [APFPM] term at constant F, it is also true that the [PEPM] term in its own right discriminates much more strongly between highly incompatible elements than does the [APFPM] term. Hence, the previous conclusions (1) (3) and (4) remain valid and (4) is in fact strengthened (see ref. 14). Conclusion (2), however, is not valid as stated. The effective bulk residue–bulk liquid distribution coefficient is D^*. The PEPM process does not strip incompatible elements from the residue with the high efficiency of the APFPM or CAPFPM processes.

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1. Gast, P W *Geochim cosmochim Acta* **32**, 1057–1086 (1968)
2. Shaw, D M *Geochim cosmochim Acta* **34**, 237–243 (1970)
3. Langmuir, G H, Bender, J F, Bence, A E, Hanson, G F & Taylor, S R *Earth planet Sci Lett* **36**, 133–156 (1977)
4. Oxburgh, E R & Turcotte, D L *J. geophys Res* **73**, 2643–2661 (1968)
5. O'Hara, M J, Saunders, M J & Mercy, E L P *Phys Chem Earth* **9**, 571–604 (1975)
6. Thompson, R N, Morrison, M A, Hendry, G L & Parry, S J *Phil Trans R. Soc* **A310**, 549–590 (1984)
7. Schilling, J G *Nature* **242**, 565–571 (1973)
8. O'Hara, M J *J Earth Sci Leeds* **8**, 219–224 (1971)
9. O'Hara, M J *Nature* **253**, 708–710 (1975)
10. Oxburgh, E R & Parmentier, E M *J geol. Soc.* **133**, 343–355 (1977)
11. Presnall, D C & Helsley, C E *Phys Earth planet Int* **29**, 148–160 (1982)
12. Presnall, D C & Hoover, J D *EOS* **45**, 1131 (1984)
13. Rhodes, J M & Sparks, J W *Proc. ISEM Conf Open Magma Systems* (eds Dungan, M A, Grove, T L & Hildreth, W) 135–137 (Inst Study Earth & Man, Dallas, 1984)
14. O'Hara, M J *J geophys Res* (in the press)

Upper mantle heterogeneities and dynamics

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The width of geochemical and residual elevation anomalies along the Mid-Ocean Ridge System decrease as both the distance between migrating ridge segments and related hotspots and the local geochemical dispersion in the anomalies increase. These observations provide further evidence that flows of mantle plumes in the upper mantle are deflected by migrating ridge sinks once they override hotspots.

THE problem of mantle heterogeneities is related closely to that of mantle dynamics[1]. Two extreme models are being pursued currently: one hypothesis considers the dispersion of randomly distributed domains rich in incompatible trace elements, passively embedded in a convecting mantle matrix relatively depleted in such elements and related radiogenic isotopes[2–5]. The second considers relatively fixed sources of incompatible element-rich domains, dynamically buoyant, rising as plumes or chains of blobs beneath hotspots which interact and mix with the incompatible element-depleted asthenosphere normally feeding migrating ridges[6–10]. Thus, migrating ridges act as sinks and mantle plumes as sources in this model[11].

We have shown recently in Galapagos[12–14] and South Atlantic[15] regions that ridge segments, once centred on a hotspot but since migrated away as far as 850 km, continue to sense and accrete material with hotspot geochemical signatures. Geochemical anomalies may also have decreased in length, local variability may have increased and the associated ridge elevation anomalies may have decreased as the distance from such ridge segments to nearby hotspots has increased. These observations provide additional evidence for a connecting channel which may develop and extend between the hotspot (the source of large-ion lithopile element (LILE)-rich material) and the migrating ridge (the sink), as proposed initially by Morgan[11] and Vink[16] purely on the basis of the tracks left by anomalous constructional volcanism on the sea floor. We also suggested that the flow of plume material towards the migrating ridge-sink becomes discontinuous, perhaps schlieren-like[15].

Here, I extend the testing of this hypothesis by considering more quantitatively how the width of these geochemical and residual elevation anomalies along the ridge crest vary either with distance from migrating ridges to nearby related hotspots, or the time since these ridge segments were located on such hotspots, or current spreading rates. On the basis of these empirical observations and very simple theoretical considerations, I then examine the consequences of this model in predicting the probable geometric pattern and evolution of the connecting flow channels, mixing conditions and constraints on mantle plume fluxes.

Methodology and results

Four major along-ridge geochemical and depth profiles were used as the database (Fig. 1). These include the Galapagos Spreading Centre (83–102° W)[12–14], the North Atlantic from 79 to 28° N[17,18], the South Atlantic from 0° to 47° S[15] and the Gulf of Aden (42–57° E; refs 19, 20 and J.-G.S., unpublished data). The four profiles combined represent about 15,430 km of ridges from the 60,000–70,000-km-long Mid-Ocean Ridge system. The average sample interval is 42 km ± 46 (1 s.d.). These four profiles comprise 11 recognizable anomalies whose widths are also shown in Fig. 1. The anomaly widths were compiled regardless of whether they represent long regular gradients, as for the ridge-centred hotspots such as Iceland, the Azores and Afar, or irregular spikes with large local dispersion, as for the more distant hotspots such as in the South Atlantic (Fig. 1). Of these 11 anomalies, 9 can be linked readily to a nearby hotspot known or suspected to have influenced the accreting crust at the ridge axis in these regions. Two could not (the 45° N anomaly in the North Atlantic and the short wavelength anomaly in the Gulf of Aden at 45.5° E). These two cases require detailed consideration which will be treated elsewhere with future tests of our model. Table 1 tabulates these geochemical relationships, including their distance to known or suspected hotspots, and the time since these ridge segments were centred on the ridge using plate-reconstructions in the framework of fixed hotspots[21–24]. I have also included in Table 1 corresponding current spreading rates taken from ref. 25 and the corresponding maximum residual elevation anomalies above the 2,900-m reference depth of normal ridges suggested by the compilation of Vogt[26] and Anderson *et al.*[27]. Whenever possible, I have attached in Table 1 probable errors associated with these parameters, such as uncertainties related to hotspot locations or plate reconstructions, major fracture zone offsets over the anomalies and sampling coverage and density.

Figure 2 shows that there is a good inverse correlation between the geochemical anomaly-width (W) and the distance (y) from the centre of these anomalies to that of the hotspot suspected or known to be linked to these anomalies (Fig. 2*a*), or with time (t) since these ridge segments were centred over these hotspots (Fig. 2*b*). For reference, these two correlations may be expressed by the empirical relations $W = (1{,}010 - 24.5\ (y)^{1/2})$ and $W = (992 - 53.6\ (t)^{1/2})$ respectively, with W and y in km and t in Myr, with correlation coefficients r^2, respectively, 0.85 and 0.89. The somewhat large deviation of the Jan Mayen hotspot from the W versus y reference curve may reflect the uncertainty in the exact location of the ridge axis on the platform and the location of this hotspot. I chose it arbitrarily to be centred on Jan Mayen Island, but this need not necessarily be the case[28]. The residual ridge-elevation anomaly (ΔE) and associated geochemical anomaly-width also decreases sympathetically (Fig. 3*a*). A linear relation $\Delta E = -0.34 + 3.13 \times 10^{-3}\ W$, with ΔE and W in km, can be used as reference (with the Azores point excluded) or $\Delta E = -0.040 + 2.47 \times 10^{-3}\ W$ (with the Azores point included) with $r^2 = 0.66$ and 0.54, respectively. The two intercepts are, within the uncertainties, close to zero, as expected for normal ridges without geochemical anomalies ($W = 0$) and normal elevation (that is, $\Delta E = 0$, or a reference depth of 2.9 km adopted from ref. 26). The empirical relationship with a linear

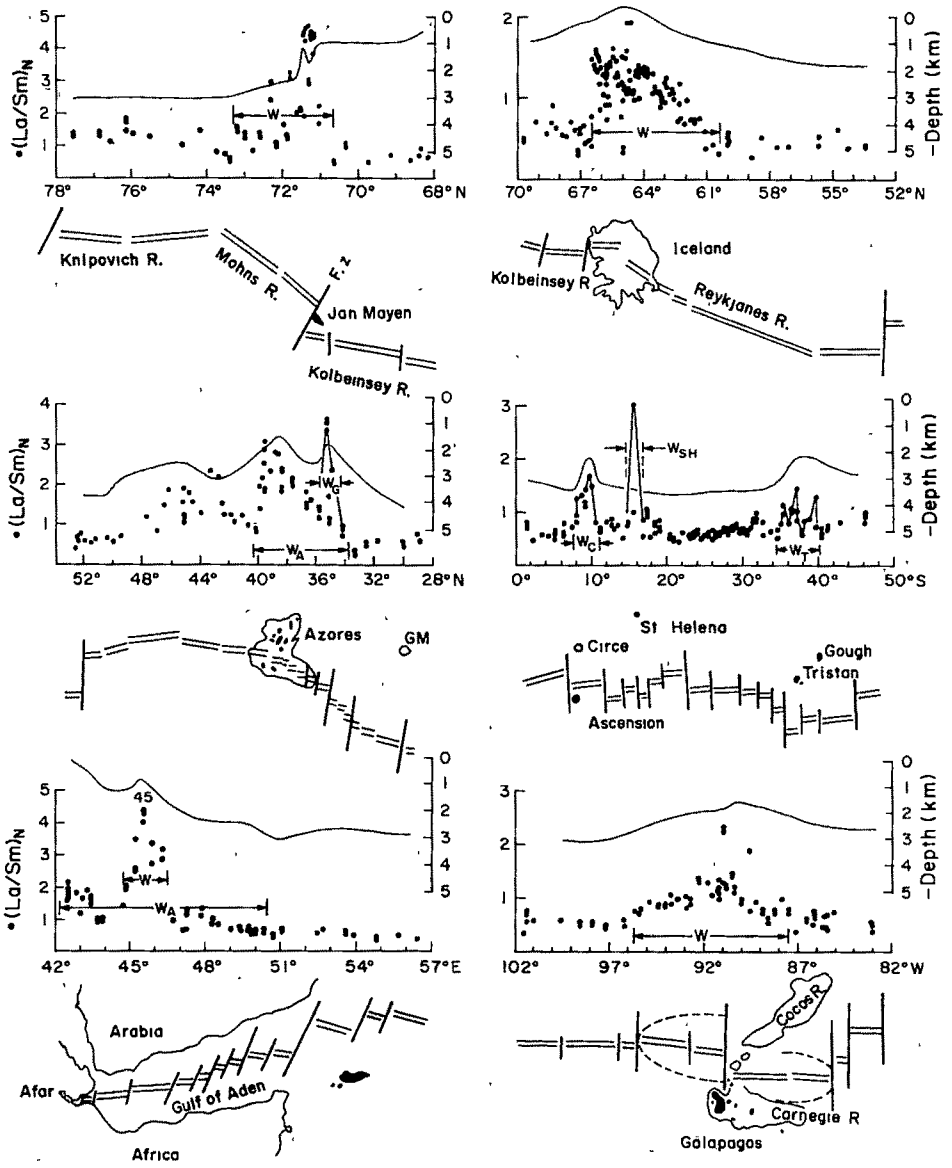


Fig. 1 La/Sm database used for determining the width (W) of geochemical anomalies along ridge axes near hotspots (left scales). Sources of profiles are: Mid-Atlantic Ridge profiles 78–71° N, 71–28° N and 0–47 °S from refs 18, 17 and 15, respectively; Galapagos 102–82° W from ref. 12; Gulf of Aden 42–57° E (from J.-S.G., manuscript in preparation). La/Sm_N is the La/Sm ratio normalized to chondrites; solid-line profiles represent the depth of sample recovery on the ridge axis, smoothed out with a method of moving average of ±100 km from each station. These depth profiles approximate the elevation of the ridge axis (see Table 1 for more exact values).

Table 1 Data used to construct Figs 2 and 3

Ridge anomaly	Associated hotspot	Hotspot location	Hotspot symbol*	Anomaly width (W) (km)	Residual† elevation (ΔE) (km)	Distance hotspot to anomaly (y) (km)	Overriding time (Myr)	Full spreading rate (S) (cm yr^{-1})
North Atlantic								
71°–73° N	Jan Mayen	71°10′ N, 8°0′ W	JM	668±115	2.2±0.2	75	—	1.66
60°–66° N	Iceland	65°0′ N, 19°0′ W	IC	923±210	3.4±0.2	0	0	1.90
41°–50° B	? (45° N)	—	—	975± 62	0.8±0.3	—	—	2.37
33°–40° N	Azores	39°38′ N, 29°45′ W	AZ	1,904±100	1.8±0.3	0	0	2.46
34°–36° N	Great Meteor	28°0′ N, 33°0′ W, 29°0′ N, 29°0′ W	GM	323±100	1.1±0.2	835–897	70	2.38
South Atlantic								
7°–11° S	Circe (Ascension)	8°15′ S, 9°20′ W	C	393±108	1.2±0.2	444	190	3.87
15°–16° S	St Helena	16°0′ S, 5°45′ W	SH	208± 96	0±0.3	811	190	3.93
35°–40° S	Tristan	37°15′ S, 12°18′ W	T	565±100	0.7±0.1	388–444	80	3.85
Red Sea/Gulf of Aden								
42°–51° E	Afar	11°40′ N, 42°30′ E	AF	1,000±145	2.9±0.2	0	0	1.80–2.02
45°–46° E	? (45.5° E)	—	—	159± 45	2.1±0.2	—	—	1.88
Equatorial Pacific								
95°–87° W	Galapagos	0°42′ S, 91°30′ W	G	880±124	1.3±0.1	213–296	7.5	6.02

* Used in Figs 2, 3. † Maximum residual elevation of geochemically anomalous ridge segment over and above a normal depth of 2.9 km adopted from ref. 26, using data from ref. 26 and modified from my own observations during dredging.

best fit forced through the origin and the Azores point included is $\Delta E = 2.42 \times 10^{-3}$ W, with $r^2 = 0.87$. Vogt[26] has shown that the residual ridge anomaly located on or near hotspots is inversely related to spreading rates. Figure 3*b* shows that the residual ridge elevation anomaly is also related to the distance of these anomalous ridge segments to their corresponding hotspot. A possible empirical relationship is $\Delta E = 2.7 - 7.7 \times 10^{-2}(y)^{1/2}$ or $\Delta E = 3.0 - 9.2 \times 10^{-2}(y)^{1/2}$, with ΔE and y in km and r^2 of 0.75 and 0.87, with or without the Azores point, respectively. The possible departure of the Azores hotspot from the correlation involving the residual ridge elevation anomaly may be related to the fact that this hotspot has become ridge-centred to its present position only 9 Myr ago, judging from kinematic plate reconstructions[21-24], as well as geochemical results from the IPOD Leg 82 sampling grid in the region[29]. The Mid-Atlantic Ridge segment just north of the Azores passed over this hotspot once before, ~38 Myr ago[21-25]. Finally, I note that there is apparently no correlation between the anomaly-width and present spreading rates, although the range of spreading rate is limited mostly to 0.8–2 cm yr^{-1} half-rate, with one exception (Galapagos, 2.8–3.1 cm yr^{-1}) (Fig. 2*c*).

The above correlations suggest that as the ridge migrates away from the hotspot, both the width of the geochemical anomaly and associated anomalous elevation caused by the hotspot decrease progressively with time. The spreading rate does not seem significantly influential, at least over the small range so far investigated.

These regularities suggest strongly that some connection exists between hotspots and migrating ridge segments which are anomolous geochemically and in residual elevation. This evidence provides further support for the development of a lateral flow, maintained between these migrating ridge sinks and corresponding mantle plumes, since the time when these ridges were initially centred on these hotspots[15].

Modelling

Ridge-centred hotspot case. We have proposed previously that along-ridge geochemical gradients about ridge-centred hotspots can be modelled by a binary mixing model comprising as end members the LILE-rich blobs and the LILE-depleted asthenosphere[7-10]. The model requires that the blob delivers material at a rate independent and in excess of the rate of accretion by sea-floor spreading over the blob; the plume tends to elevate the region. The model also assumes that in the absence of a plume, accretion is passive and results simply from the divergence of the plates. The excess of blob material tends to pile up and form thicker crusts and is also accommodated by some flow outward along the ridge which acts as a sink. The mixing proportion (P) of blob material relative to the total mass of material accreting per unit ridge-length could decrease from unity over the plume (providing that no dilution with the depleted asthenosphere took place while travelling through it, or that the melting-zone width across the ridge is smaller than that of the blob) to zero at the end of the gradient where plume material has been completely used up by the accretion process (Fig. 4). We have shown previously that by estimating the trace-element content and the isotopic composition of the two end members (or Sm content and La/Sm ratios), the variation of P along the ridge axis (taken as the x direction) can be determined readily from the isotopic ratio variation (or La/Sm; see refs 12, 13). We can also estimate the minimum flux of the blob (Q), assuming that the gradient in La/Sm represents a quasi-steady state, namely:

$$Q = 2\int_{x=0}^{x=L} H(x)\, S(x)\, P(x)\, dx \qquad (1)$$

Observations suggest that the crustal thickness (H) and the full spreading rate (S) may both be functions of the distance (x) from the blob. In the evaluation of the physics of the process, it is probable that $H(x)$ would be dependent on $S(x)$. To illustrate the point simply, however, I assume in Fig. 4 that: (1) the blob is a point source at $x = 0$; (2) the two gradients are

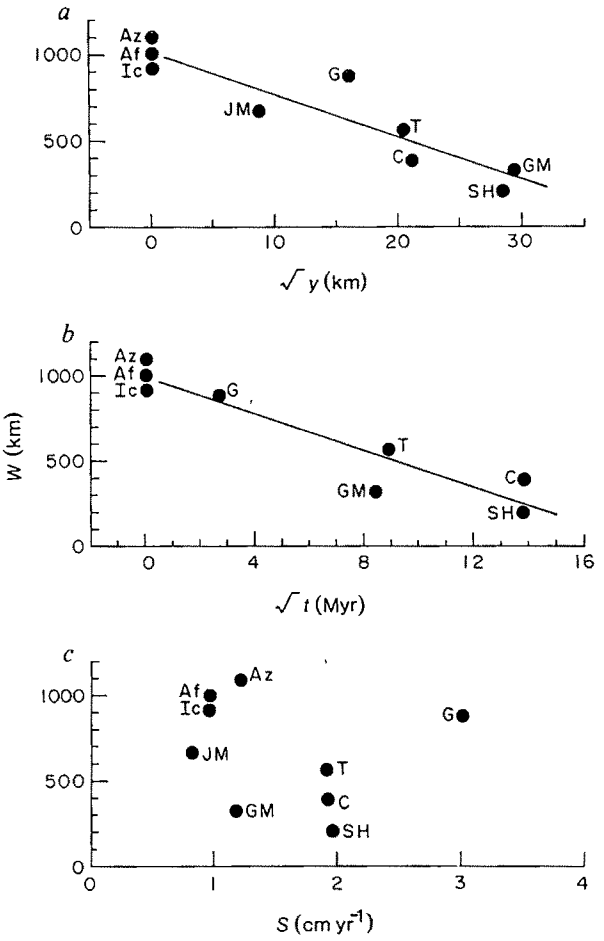


Fig. 2 Geochemical anomaly width (W) versus· *a*, square root of present distance (y) of associated hotspot to the geochemical anomaly on migrating ridge (determined from Fig. 1); *b*, square root of time (t) since the ridge was centred on associated hotspot (determined from refs 22–24); and *c*, half spreading rates (S) (determined from ref 25). All values used, including symbols used for hotspot names, are listed in Table 1. Note the good best-fit inverse linear correlation of W with $y^{1/2}$ and W with $t^{1/2}$ with correlation coefficients r^2 of 0.85 and 0.89 respectively, but lack of correlation of W with S.

placed symmetrically about the blob at $x = 0$, hence the factor two in equation (1); (3) the two end-member materials have essentially the same density, thus $P(x)$ represents volumetric proportions and Q is in km^3 yr^{-1}, if S is in km yr^{-1}; (4) H and S are constants independent of (x); (5) P varies linearly with x and is given by $P = 1 - (x/L)$, with $(0 \leq P \leq 1$; Fig. 4). Under these conditions, integration of equation (1) gives

$$Q = HSL = HSW/2 \qquad (2)$$

or

$$W = 2Q/HS \qquad (3)$$

where W is twice the length of gradients distributed symmetrically about the blob.

Thus, for ridge-centred blobs $W = 2Q/HS$ and is directly proportional to the blob flux and inversely proportional to S and H. For a given plume flux and constant crustal thickness, W or the length of the gradient L, should decrease with increasing spreading rate from one ridge-centred hotspot to another.

In reality, equation (1) must be integrated separately for each ridge-centred hotspot situation, with different boundary conditions and appropriate $H(x)$, $S(x)$ and $P(x)$ functions. In such cases, the relation of Q with L or W may be of a higher order than indicated in equations (2) or (3). Minimum estimates of plume fluxes can be made by this method and will be reported on in detail elsewhere.

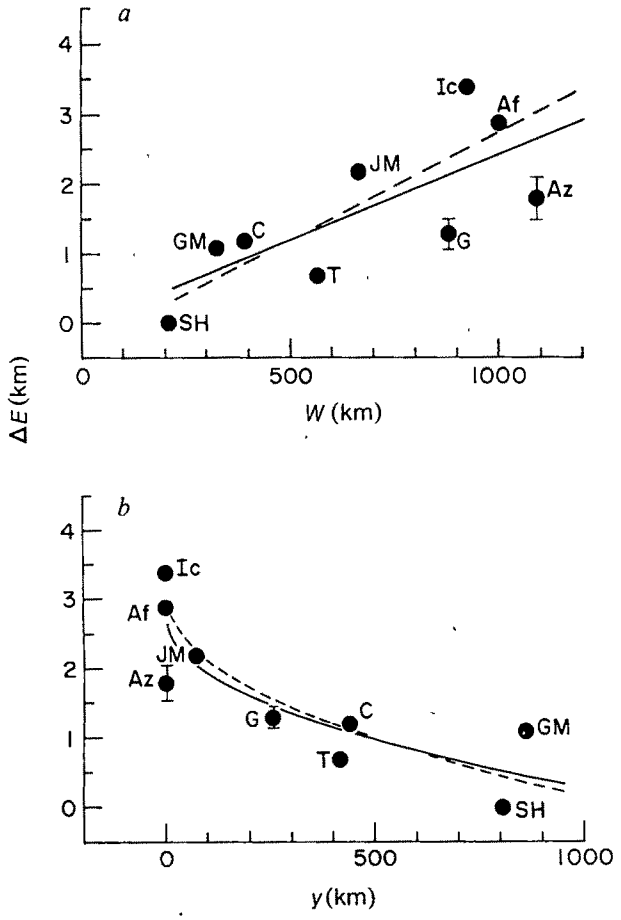


Fig. 3 Residual elevation (ΔE) of ridge axis over geochemical anomalies versus: *a*, geochemical anomaly width W (solid and dashed lines are linear best-fits including and excluding the Azores, respectively and the correlation coefficients are respectively, 0 54 and 0.66); *b*, distance (y) of hotspot to the geochemical anomaly on the migrating ridge (dashed and solid curves are fitted to data with and without the Azores respectively; see text for empirical relationships). Residual elevation ΔE is the maximum elevation observed along the geochemical anomaly above the 2,900 m-depth base line of normal ridge axes adopted from ref. 26 (that is, ridge segments not influenced by hotspots and without any geochemical anomalies). All values used are listed in Table 1.

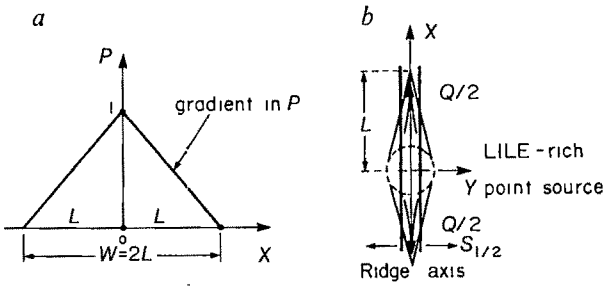


Fig. 4 *a*, Steady state, subcrustal ridge-centred mantle-plume flow model considered in text. Diamond-like figure delineates idealized boundary of plume flow which along x feeds in part of the spreading ridge axis (heavy parallel line) with LILE-rich material. *b*, Corresponding variation with x of the fraction (P) of LILE-rich plume material entering the mix with LILE-depleted asthenosphere to form the new oceanic lithosphere. The two vectors represent integrated plume flux $Q/2$ along the x direction when a steady state is reached with new lithosphere formation by spreading along the ridge axis (sink).

Finally, I note that Vogt[26] has modelled independently fluxes for along-ridge subcrustal pipe flows from hotspots on the basis of topographic gradients. These fluxes[26] should not be confused with the flux in my equation (1) which refers only to material with plume signature. The latter could be only a fraction of the total flux taking place along the ridge proposed by Vogt's model[26].

Off-ridge hotspot case. I can now extend the steady-state, ridge-centred hotspot model to the situation where there are fixed off-ridge hotspot sources but migrating ridges. Here the flux Q represents the rate at which plume material is being discharged at the mouth of the lateral channel connecting the off-ridge hotspot to the migrating ridge. I refer to this discharge flux as Q_d. Any excess of plume-derived material would be deflected along the ridge axes on either side of this intersection and be used up progressively by the accreting process. As I have determined empirically that W is related inversely to the distance of the ridge to the hotspot (y) (or inversely related to the time (t) elapsed since the migrating ridge was over the hotspot), it follows from equation (3) that the Q_d of plume-related material reaching the migrating ridge may, to a first approximation, be related inversely to the distance y ($Q_d \propto 1/y$) (or inversely related to t). In other words, the amount of plume material (or mixture of plume and asthenospheric materials) reaching the migrating ridge should decrease progressively with time and as the migrating ridge becomes more distant from the hotspot, until it becomes essentially intraplate. Eventually, our steady-state model will no longer apply because of the lack of excess plume flow; only a trickling of plume material will reach the ridge with very inefficient mixing[15]. Here, (for example, St Helena) W may only approximate the width of the lateral connecting channel. Of course, I do not suggest that by combining all the hotspot-migrating ridges so far studied, an inverse relationship should exist between the plume discharge rates Q_d and y or t. This stems from the fact that the blob size and total plume flux Q may vary from one hotspot to another, or with time for a single hotspot. As I have noted previously, the spreading-rate variation with x, which may also affect $H(x)$, will differ from one hotspot–ridge system to another.

Flow pattern

Two simple types of arguments provide additional support for suggesting in plan form in the upper mantle a relatively narrow flow channel from the off-ridge hotspot to the migrating ridge.

The first method is based on the comparison of the spatial geochemical variation and derived P values observed with those predicted in plan form from a radially zoned mantle-plume flow tapped passively by the migrating ridge (Fig. 5*a*). This approach is particularly amenable to tests if large fracture zone offsets are present, for example, the Galapagos[12–13], thus causing major discontinuities in distances from the ridge axis to the hotspot and predicting discontinuities in the geochemical variations and related P values. Here, it was shown geochemically that such a radially zoned plume flow model is not viable; instead, a northward channelled plume flow toward the ridge is more appropriate, as suggested independently by Morgan[11] on the basis of constructional volcanism and plate kinematics. The same kind of argument can be used for the Tristan configuration, where the Mid-Atlantic Ridge stations located nearest to the Tristan hotspot are not the most enriched in La/Sm (Fig. 1). This test, however, becomes more difficult to apply to the case of the most distant St Helena, Circe and Great Meteor hotspots, because of the large local dispersion and poor mixing conditions observed in the related along-ridge geochemical anomalies and the lack of major ridge offsets along these segments.

In the second type of test, I consider only the variation of W with t or y to the hotspot, regardless of the form of the variation $P(x)$ in the anomaly (Fig. 5*b*, *c*). Figure 5*b* shows again a model which considers in plan form an outward radial plume flow, extending to a radius R and its passive tapping by the migrating ridge above. The radial variation of the P value need not be specified. The circumference limit at R can be considered physically either as the location where the plume is so diluted with

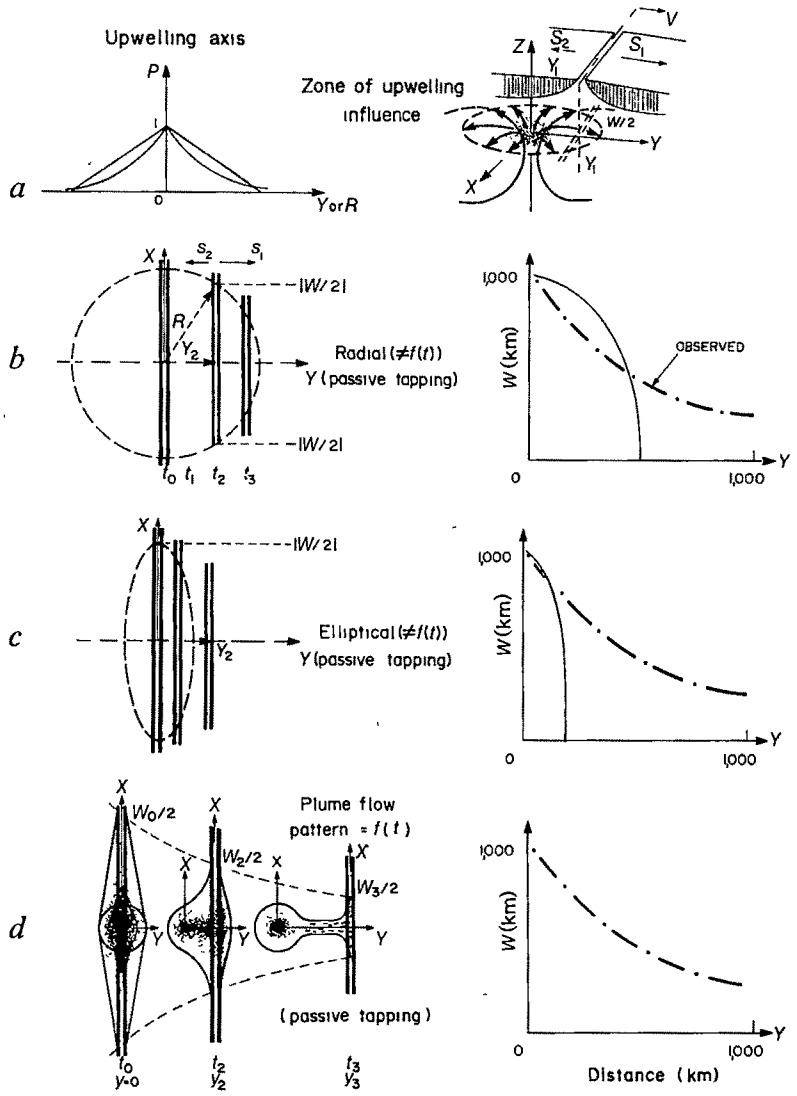


Fig. 5 Models of plume-flow (source) migrating ridge (sink): The vectors S_1 and S_2 are the half-spreading rates relative to the ridge axis; V is the component of the absolute motion of the migrating ridge along the spreading direction and relative to the hotspot which is considered fixed in space. The mixing fraction P of LILE-rich material is specified only in model *a*, where it decreases regularly outward from the centre of the plume. In model *b*, the zone of plume influence detected and passively tapped by the migrating ridge is circular and independent of time, whereas in *c* it is elliptical. Both models *a* and *b* predict a variation of W on the ridge with y which is concave downward, instead of upward as observed empirically in Fig. 2. Model *d* considers a plume-flow pattern variable in time and influenced by the migrating ridge, hence changing pressure gradients. The plume-flow configuration is shown for three different times. At t_0, the plume is ridge-centred, the ridge is elevated anomalously, the plume flow is mostly along the x-axis, and P varies regularly with x (same model as in Fig. 4, for example, Iceland). At time t_3, the plume flow is now channelled toward the more distant ridge, W on the ridge is minimal and the elevation is now essentially normal, mixing is irregular and the heterogeneities are schlieren-like (for example, St Helena-Mid Atlantic Ridge). At time t_2, an intermediate case, the plume flow is skewed both toward and along the migrating ridge which acts as a sink (for example, Galapagos).

the depleted asthenosphere that it can no longer be detected or that the radial plume panache is returning down into the mantle and at the radius R the return flow is deeper than the partial melting zone where lavas used in the sampling take source (Fig. 5*b*). As the ridge is migrating along the y direction over the zone of influence of the plume (that is, when $y < R$), the length of the ridge over which material with plume signature can be detected (that is, W) decreases as expected from the empirical relations established in Fig. 2. The predicted form of the variation of W with y, however, is concave downward, rather than upward as actually observed. This is also the case if the zone of upwelling plume influence is elliptical as a result of initial down-flow along the ridge when it was centred over the hotspot (Fig. 5*c*). These two radial models, where the shape of the zone of plume influence in the upper mantle is considered to be essentially constant and independent of time, must therefore be rejected. A more dynamic situation is required to match the empirical variation of W with t or y (Fig. 5*d*). The plume-flow pattern about the hotspot must change with t as the plume-source/ridge-sink geometric configuration and associated resulting pressure gradients change progressively from ridge-centred to a near-ridge and finally to completely off-ridge.

We believe that the model of a highly skewed plume flow along the ridge when the hotspot was ridge-centred and the lateral flow which developed subsequently and continued to feed the plume flow along the ridge as it begins to migrate away from the hotspot, can explain satisfactorily the observed variation of W with t or y; the model is sketched in Fig. 5*d*. As long as the discharge rate at the mouth of the channel exceeds that of the accretion process per unit ridge length at this intersection, some flow will take place along the ridge and a gradient may develop if mixing conditions are regular. As the distance of the ridge to the hotspot increases with t, the plume flow along the lateral channel and the ridge axis decreases as does the width of the anomaly. The ridge crest also subsides and the mixing conditions become more irregular; considerable dilution with the surrounding depleted asthenosphere seems to occur along the channel, perhaps by entrainment and shearing associated with return flows toward the ridge in the upper mantle. Figure 5*d* illustrates how W may change with time, assuming that the empirical relationship observed from the pool of ridge-hotspot configuration so far studied is directly applicable to a single hotspot-ridge situation. W would decrease rapidly as soon as the migrating ridge leaves the hotspot and the rate of decrease slows down as y increases.

To conclude, I emphasize that, in general, the regularities observed between the geochemical and elevation anomalies along the ridge crest and their position relative to hotspots are clearly not random and are probably not coincidental. Thus, I believe that the model of hotspot-source migrating-ridge sink and a connecting flow channel deserves further consideration and testing. The regularities noted should also be taken into consideration by proponents of the alternative stochastic plum-pudding model[4,5]. With these modifications, these two extreme models need not be necessarily mutually exclusive.

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1. Bougault, H., Cande, S. C., Schilling, J.-G., Turcotte, D. L. & Olson, P. *Nature* **305**, 278–279 (1983).
2. Richter, F. M. & Ribe, N. M. *Earth planet. Sci. Lett.* **43**, 212–222 (1979).
3. Olson, P., Yuen, D. A. & Balsiger, D. *J. geophys. Res.* **89**, 425–436 (1984).
4. Davies, G. F. *Nature* **290**, 208–213 (1981).
5. Sleep, N. H. *J. geophys. Res.* **89**, 10029–10041 (1984).
6. Morgan, W. J. *Nature* **230**, 42–43 (1971).
7. Schilling, J.-G. *Nature* **242**, 565–571 (1973).
8. Hart, S. R., Schilling, J.-G. & Powell, J. L. *Nature* **246**, 104–107 (1973).
9. Sun, S.-S., Tatsumoto, M. & Schilling, J.-G. *Science* **190**, 143–147 (1975).
10. Schilling, J.-G. & Noe Nygaard, A. *Earth planet. Sci. Lett.* **24**, 1–14 (1974).
11. Morgan, W. J. *J. geophys. Res.* **83**, 5355–5360 (1978).
12. Schilling, J.-G., Kingsley, R. & Devine, J. D. *J. geophys. Res.* **87**, 5593–5610 (1982).
13. Verma, S. P. & Schilling, J.-G. *J. geophys. Res.* **87**, 833–856 (1982).
14. Verma, S.P., Schilling, J.-G. & Waggoner, D. G. *Nature* **306**, 654–657 (1983).
15. Schilling, J.-G., Thompson, G., Kingsley, R. & Humphris, S. *Nature* **313**, 187–191 (1985).
16. Vink, G. E. *J. geophys. Res.* **87**, 9949–9959 (1984).
17. Schilling, J.-G. *et al. Am. J. Sci.* **283**, 510–586 (1983).
18. Neumann, E.-G. & Schilling, J.-G. *Contrib. Miner. Petr.* **85**, 209–223 (1984).
19. Schilling, J.-G. *Trans. Am. geophys. Un.* **58**, 535 (1977).
20. Schilling, J.-G., Vollmer, R. & Bergeron, M. *Trans. Am. Geophys. Un.* **61**, 405 (1980).
21. Morgan, W. J. *The Sea* **7**, 443–487 (1981).
22. Morgan, W. J. *Tectonophysics* **94**, 123–139 (1983).
23. Duncan, R. A. *Tectonophysics* **74**, 29–42 (1981).
24. Duncan, R. A. *J. geophys. Res.* **89**, 9980–9990 (1984).
25. Minster, J. B. & Jordan, T. H. *J. geophys. Res.* **83**, 5331–5354 (1978).
26. Vogt, P. R. *Earth planet. Sci. Lett.* **29**, 309–325 (1976).
27. Anderson, R. N., McKenzie, M. & Sclater, J. G. *Earth planet. Sci. Lett.* **18**, 391–407 (1973).
28. Waggoner, D. G., Vollmer, R. & Schilling, J.-G. *Trans. Am. geophys. Un.* **62**, 423 (1981).
29. Rideout, M. L. & Schilling, J.-G. *IPOD Initial Rep.* **82** (in the press).

Hypervariable 'minisatellite' regions in human DNA

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The human genome contains many dispersed tandem-repetitive 'minisatellite' regions detected via a shared 10–15-base pair 'core' sequence similar to the generalized recombination signal (χ) of Escherichia coli. *Many minisatellites are highly polymorphic due to allelic variation in repeat copy number in the minisatellite. A probe based on a tandem-repeat of the core sequence can detect many highly variable loci simultaneously and can provide an individual-specific DNA 'fingerprint' of general use in human genetic analysis.*

DNA POLYMORPHISMS have revolutionized human genetic analysis and have found general use in antenatal diagnosis[1], mapping of human linkage groups[2,3], indirect localization of genetic disease loci by linkage[2,4,5] and analysis of the role of mitotic nondisjunction and recombination in inherited cancer[6–9]. Single-copy human DNA probes are used normally to detect restriction fragment length polymorphisms (RFLPs), most of which result from small-scale changes in DNA, usually base substitutions, which create or destroy specific restriction endonuclease cleavage sites[10,11]. As the mean heterozygosity of human DNA is low (~0.001 per base pair)[10–12], few if any restriction endonucleases will detect a RFLP at a given locus, although the probability of detection is improved for enzymes such as *Msp*I and *Taq*YI which contain the mutable CpG doublet in their recognition sequence[13]. Even when detected, most RFLPs are only dimorphic (presence or absence of a restriction endonuclease cleavage site) with a heterozygosity, determined by allele frequencies, which can never exceed 50% and which is usually much less. As a result, all such RFLPs will be uninformative in pedigree analysis whenever critical individuals are homozygous.

Genetic analysis in man could be simplified considerably by the availability of probes for hypervariable regions of human DNA showing multiallelic variation and correspondingly high heterozygosities. The first such region was isolated by chance by Wyman and White[14] from a library of random segments of human DNA. The structural basis for multiallelic variation at this locus is not yet known. Subsequently, and again by chance, several other highly variable regions have been discovered near the human insulin gene[15], α-related globin genes[16–18] and the c-Ha-*ras*-1 oncogene[19]. In each case, the variable region consists of tandem repeats of a short sequence (or 'minisatellite') and polymorphism results from allelic differences in the number of repeats, arising presumably by mitotic or meiotic unequal exchanges or by DNA slippage during replication. The resulting minisatellite length variation can be detected using any restriction endonuclease which does not cleave the repeat unit and provides for such loci a set of stably inherited genetic markers.

We have described previously a short minisatellite comprised of four tandem repeats of a 33-base pair (bp) sequence in an intron of the human myoglobin gene (ref. 20; Fig. 1). The 33-bp repeat showed some similarity in sequence to three other hypervariable human minisatellites characterized previously and on the basis that the myoglobin minisatellite was flanked by a 9-bp direct repeat characteristic of the target site duplications generated by transposable elements, we suggested that this minisatellite and some other hypervariable regions were related via transposition. We show here that the myoglobin 33-bp repeat is indeed capable of detecting other human minisatellites, some of which are highly polymorphic. These regions, however, are not related by transposition, but instead share a common short 'core' sequence in each repeat unit, which in turn provides a powerful probe for hypervariable regions.

Probe for variable human DNA

A pure repeat probe was prepared from the human myoglobin minisatellite by purification of a single 33-bp repeat element followed by head-to-tail ligation and cloning of the resulting polymer into pUC 13 (ref. 21; Fig. 1). Cleavage of one of the resulting recombinants, pAV33.7, with *Bam*HI plus *Eco*RI released a 767-bp DNA insert comprised almost entirely of 23 repeats of the 33-bp sequence.

Low stringency hybridization of this repetitive insert to human DNA digested with restriction endonuclease *Eco*RI detected numerous cross-hybridizing DNA fragments, some of which showed signs of polymorphic variation (data not shown). To improve the detection of polymorphisms, the hybridization was repeated with human DNA digested with *Hin*fI or *Hae*III, both of which cleave at a 4-bp sequence not present in the 33-bp repeat sequence and which should release minisatellites in relatively small DNA fragments whose size will reflect more closely the number of repeats per minisatellite. As shown in Fig. 2, the repetitive probe detected multiple DNA fragments in human DNA as well as the parent DNA fragment from the human myoglobin gene. The larger DNA fragments (in the range 2–6 kilobases (kb) and substantially larger than the mean DNA fragment size of ~0.3 kb in human DNA digested with *Hin*fI or *Hae*III) in particular showed variation between the three individuals examined; these variants were transmitted apparently in a Mendelian fashion, in that each polymorphic

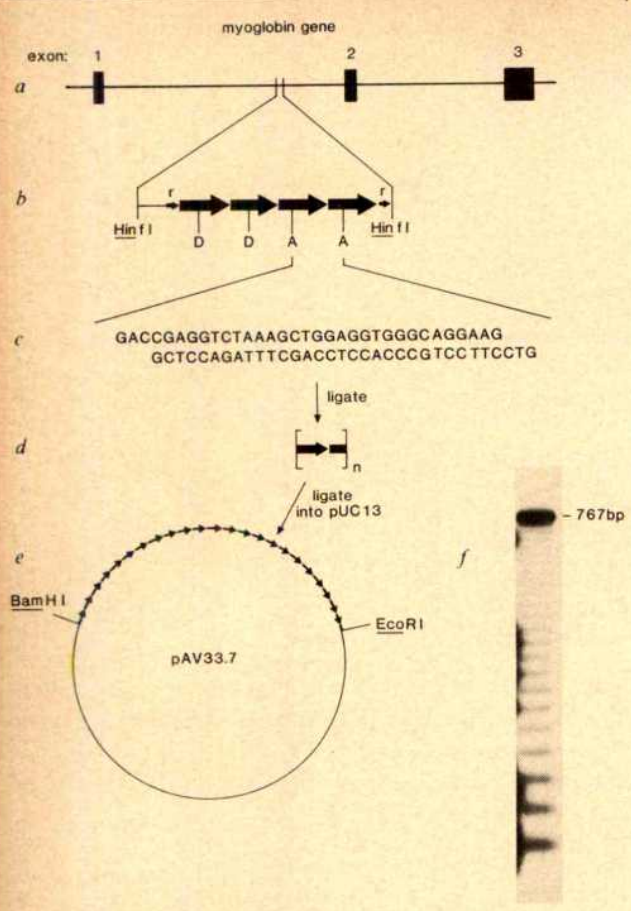


Fig. 1 Construction of a tandem-repetitive hybridization probe for 33-related DNA sequences. *a*, *b*, This probe was derived from a tandem repetitive segment of the human myoglobin gene[20]. This region, located in the first intron and comprising four repeats of a 33-bp sequence flanked by a 9-bp direct repeat (r), was isolated in a 169-bp *Hin*fI fragment, end-repaired and amplified by cloning into the *Sma*I site of pUC13 (ref. 21). *c*, A 33-bp repeat monomer was isolated by cleaving the third and fourth repeat with *Ava*II (A); a single base substitution in repeats 1 and 2 eliminates this site and creates instead a *Dde*I (D) cleavage site[20]. *d*, Ligation of the 33-bp monomer via the non-identical *Ava*II sticky ends produced a head-to-tail polymer. Polymers containing ⩾10 repeats were isolated by preparative agarose gel electrophoresis[33], end-repaired, ligated into the *Sma*I site of pUC13 and cloned in *E. coli* JM83 (ref. 21). *e*, The structure of clone pAV33.7 was confirmed by excision of the insert at the polylinker with *Bam*HI plus *Eco*RI, fill-in labelling with α-^{32}P-dCTP at the *Bam*HI site, and partial digestion with *Ava*II. *f*, Labelled partial digest products were resolved by electrophoresis on a 2% agarose gel. pAV33.7 contains 23 repeats of the 33-bp monomer contained in a 767-bp *Bam*HI/*Eco*RI fragment as shown (*e*).

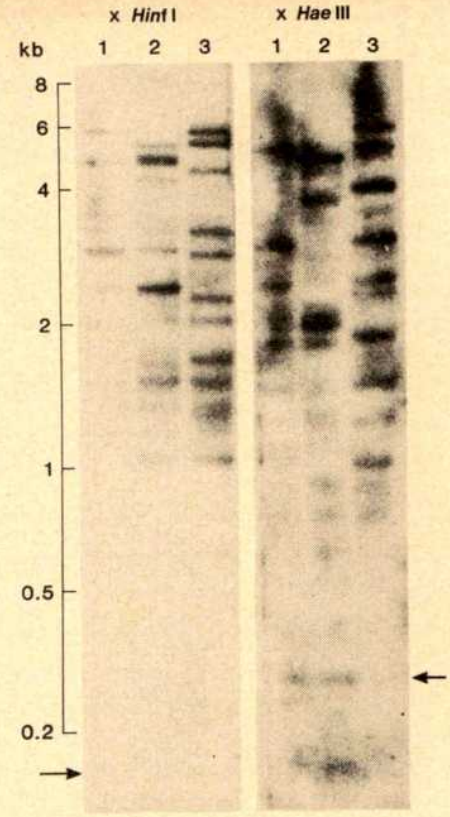


Fig. 2 Detection of multiple 33-related sequences in human DNA. 10 μg samples of DNA from individual 1 (daughter), 2 (mother) and 3 (father) were digested with *Hin*fI or *Hae*III, electrophoresed through a 1% agarose gel and transferred by blotting[35] to a Pall Biodyne membrane to prevent the loss of small DNA fragments. The membrane was hybridized in 1×SSC at 60° with dextran sulphate[36] to the insert from pAV33.7 (Fig. 1) labelled *in vitro* with ^{32}P (ref. 20). The arrowed fragment in each digest is derived from the minisatellite located in the myoglobin gene (Fig. 1); a survey of DNA from 12 individuals digested with *Hae*III showed that this myoglobin minisatellite is monomorphic (data not shown).

band in the daughter could be identified within one or other (but not both) parents. Variation was detectable in both *Hin*fI and *Hae*III digests of human DNA, consistent with polymorphism resulting from length variation of minisatellite regions.

Isolation of minisatellites

A human genomic library[20] of 10–20 kb *Sau*3A partials of human DNA cloned in phage λL47.1 (ref. 22) was screened by hybridization with the 33-bp repeat probe from pAV33.7. At least 40 strongly-to-weakly hybridizing plaques were identified in a library of 3×10^5 recombinants, consistent with the complexity of the Southern blot hybridization (Fig. 2). A random selection of eight of these positive plaques was purified (λ33.1–15) and Southern blot analysis of phage DNA showed that in each recombinant the hybridizing DNA was localized in a unique short (0.2–2 kb) region of the recombinant. Sequence analysis (Fig. 3) showed that this region in each of the eight recombinants contains a minisatellite of 3–29 tandem copies of a repeat sequence whose length ranged from 16 bp in λ33.15 to 64 bp in λ33.4. Most minisatellites contained an integral number of repeats. In λ33.6, the 37-bp repeat consisted in turn of a diverged trimer of a basic 12-bp unit. Each λ33 recombinant represented a different region of the human genome, judged by the clone-specific DNA sequences flanking each minisatellite.

Highly polymorphic minisatellites

The eight cloned minisatellite regions were located in 0.5–2.2-kb *Hin*fI DNA fragments, smaller than the clearly polymorphic 2–6-kb DNA fragments detected by pAV33.7 in *Hin*fI digests of human DNA (Fig. 2). To determine whether any of the cloned regions were also polymorphic, ^{32}P-labelled single-stranded DNA probes were prepared from suitable M13 subclones of each minisatellite and hybridized at high stringency to a panel of 14 unrelated British caucasian DNAs digested with *Hin*fI. Typical hybridization patterns are shown in Fig. 5, showing that under these hybridization conditions each probe detects a unique region of the human genome. Alleles detected by each probe are summarized in Table 1 where in each case, the most common *Hin*fI allele corresponded in size to the *Hin*fI minisatellite fragment in clones λ33.1–15, suggesting that these regions have been isolated without major rearrangement.

In the limited population sample studied, four of the eight minisatellites showed polymorphic variation. Three of the regions were highly polymorphic with between five and eight resolvable *Hin*fI fragment-length alleles detected per locus. This variation almost certainly results from variation in the repeat

Table 1 Allelic variation at individual cloned minisatellites

Clone λ33	Repeat length (bp)	Divergence %	Alleles			$4N_eu$		
			Length (bp)	No. repeats	Frequency	A	B	C
1	62	0.2 ± 0.2	3,150	40	0.04	2	3	9
			2,600	31	0.11			
			2,350	27	0.04			
			*2,300	26	0.71			
			2,190	24	0.07			
			1,950	20	0.04			
3	32	14.1 ± 2.5	*450	6	1.00	0	0	0
4	64	6.9 ± 1.5	2,280	18	0.07	2	2	5
			2,140	16	0.11			
			*2,015	14	0.43			
			1,950	13	0.36			
			1,780	10	0.04			
5	17	9.2 ± 1.9	*1,660	14	1.00	0	0	0
6	37	0.7 ± 0.4	1,800	25†	0.04	4	6	20
			1,650	21†	0.07			
			1,570	19†	0.04			
			*1,535	18†	0.43			
			1,450	16†	0.04			
			1,400	15†	0.25			
			1,350	14†	0.04			
			1,280	12†	0.11			
10	41	5.9 ± 0.6	*1,460	5	1.00	0	0	0
11	33	0.0 ± 0.0	*990	3	1.00	0	0	0
15	16	1.1 ± 0.5	1,410	41	0.50	0.3	0.3	0.3
			*1,220	29	0.50			

DNA was prepared from white blood cells[10] from 14 unrelated British caucasians (seven male, seven female), digested with *Hin*fI and Southern blot hybridized at very high stringency with probes from subcloned minisatellites as described in Fig. 5*a*, *b*. Cloned alleles whose sequences are shown in Fig. 3 are indicated (*). The divergence of each sequenced minisatellite from a hypothetical $(consensus)_n$ sequence is given as the mean percentage unique substitution divergence (±s.e.m.) to correct for variants which have diffused over more than one repeat. For example, λ33.15 shows 13 variants (10 substitutions and three deletions) but only five distinct variants over 29 repeats of a 16-bp sequence, giving a divergence from a $(consensus)_{29}$ sequence of $5/(29\times16) = 1.1\%$. The number of repeats in each allele was estimated from DNA fragment size; this estimate for alleles of λ33.6 is approximate (†) because of the trimeric nature of the λ33.6 repeat unit (Fig. 3). Approximate values of $\theta = 4N_eu$, where N_e is the effective population size and u is the rate of production of new length variants per locus per gamete, were estimated for each minisatellite from the number of different alleles (n_a) in the sample of 14 individuals by extensive population computer simulations designed to estimate the number of different, selectively equivalent alleles that could be maintained at steady-state in a population according to three recombinational models: A, random unequal crossing over between two alleles at meiosis, giving a new allele comprised of a random-length 5′ segment of one allele fused to random length 3′ segment of the second allele; B, constrained unequal exchange such that an allele mutates at random to gain or lose between one and three repeats; C, constrained slippage causing the gain or loss of only a single repeat. In each model, it is assumed as a first approximation that u is independent of the number of repeats in allele, as for the loci presented there is at most only a two-fold variation in allele length. Simulations were performed for values of θ from 0.1 to 100, using population sizes N_e from 50 to 500, and were continued for 10 N_e generations, steady state being achieved within $\sim N_e$ generations. Results from model A closely approximated the infinite allele model at $\theta < 2$, when the expected number of alleles n_a in a sample of i individuals is given by $n_a = \sum_1^{2i} (\theta/\theta + i - 1)$(ref. 31). Model C is the charge state model for which n_a has yet to be solved as a function of θ (ref. 32).

copy number in minisatellites, as alleles generally differed in length by an integral number of repeat units. In addition, longer alleles tended to hybridize with minisatellite probes more strongly than shorter alleles (see individual 1 in Fig. 5*a*), again suggesting that longer alleles contain more repeat sequence.

There is considerable variation in the level of repeat sequence homogeneity in each sequenced minisatellite region (Fig. 3). Some minisatellites (for example, λ33.3 and 5) show substantial repeat divergence, suggesting that these regions are not actively undergoing sequence homogenization by unequal exchange[23]; as expected, these regions show no polymorphic variation in repeat number (Table 1). Instead, the highly polymorphic minisatellites (λ33.1, 4 and 6) all show high repeat copy number together with substantial sequence homogeneity of repeats. In addition, base substitutions in the repeat units of hypervariable minisatellites tend to be present in more than one repeat (see, for example, λ33.15 in Fig. 3) which indicates that these minisatellites are actively and repeatedly engaging in unequal exchange, resulting in the diffusion of novel base substitutions across more than one repeat unit[23].

We used computer simulations to estimate the rate of unequal exchange needed to maintain the number of different (neutral) alleles (n_a) seen in our population sample (Table 1). Although space does not allow a detailed description of the population simulations, we find that for a population of N_e diploid individuals starting with a monomorphic minisatellite containing say 30 repeats, n_a reaches a steady state in $\sim N_e$ generations, the mean value of n_a being determined both by the parameter $\theta = 4N_eu$, where u is the rate of production of new length alleles per locus per gamete, and by the model of unequal exchange used in the population simulation. Three models have been investigated: (1) random meiotic unequal exchange between minisatellite alleles; (2) constrained sister chromatid exchange; (3) DNA slippage causing the gain or loss of a single repeat (Table 1, models A, B and C respectively). We favour the constrained exchange model because of the tendency for minisatellite base substitution variants to diffuse to non-adjacent repeats (Fig. 3), together with the tendency of different length alleles of minisatellites to differ from each other by several rather than either one or many repeat units (Table 1). For the highly polymorphic minisatellites, we estimate θ to be 2–6. Given that the effective population size N_e for human populations has been estimated at $\sim 10^4$ (ref. 24), this gives values of u, the mutation rate to a new length allele, of $\sim 0.5\text{–}1.5\times10^{-4}$ per gamete for a minisatellite ~1 kb long.

This value is higher than the base substitution neutral mutation rate in man, which from studies of non-coding DNA sequence divergence in man and higher primates has been

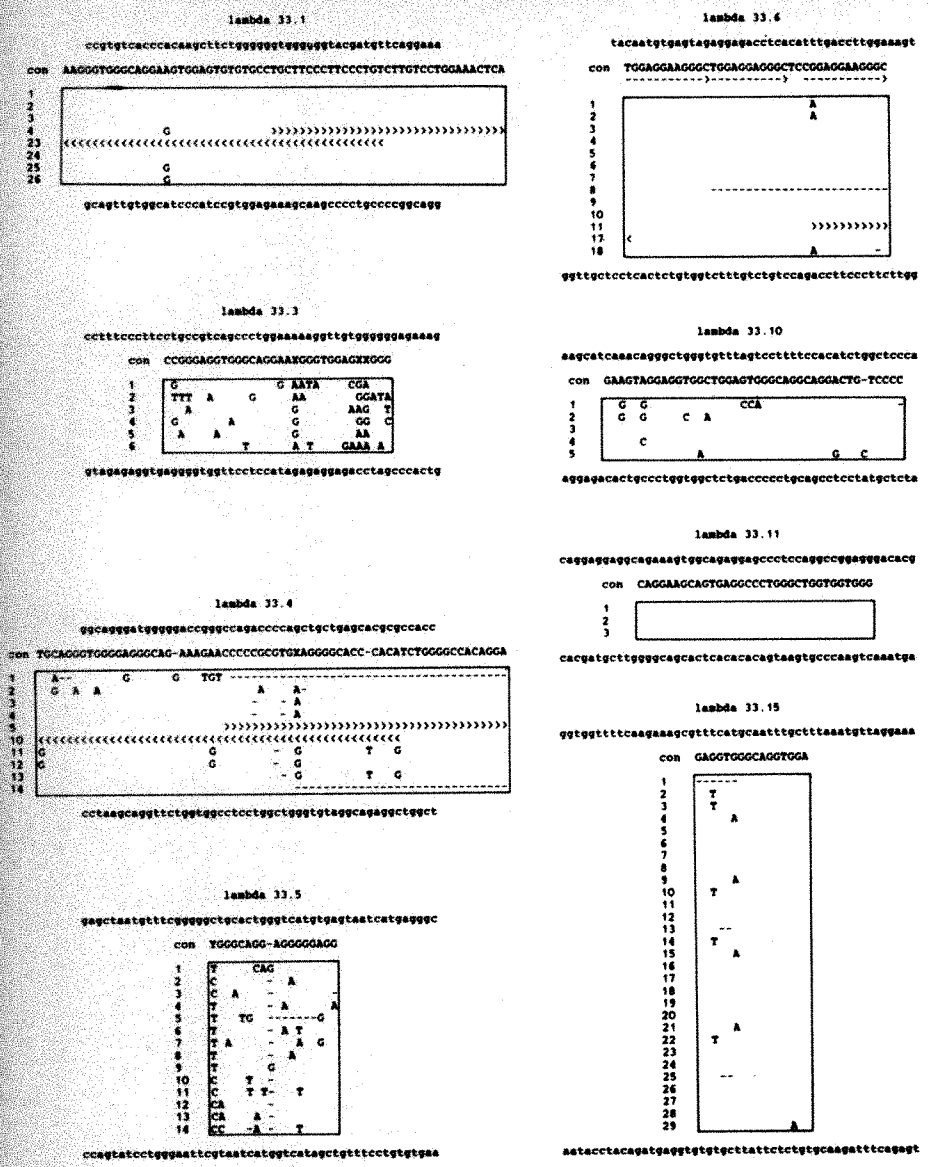


Fig. 3 Sequences of a selection of minisatellite regions detected by the myoglobin 33-repeat probe. The consensus sequence (con) of the tandem repetitive region in each of the genomic clones λ33.1–15 is shown, together with 50 bp of 5′ and 3′ flanking DNA (lower case). Differences from the consensus sequences are also shown for the individual numbered repeats (X, A or G; Y, C or T; −, missing nucleotide; »«, region not sequenced although clearly a tandem repeat of the consensus sequence, or of a close derivative of the consensus, by inspection of sequencing autoradiographs).

Methods. A library of 10–20-kb human DNA fragments cloned in bacteriophage λL47.1 (refs 20, 22) was screened by hybridization with ^{32}P-labelled pAV33.7 insert as described in Fig. 2. A random selection of eight positive plaques was purified to give recombinants λ33.1–15. Each phage DNA was digested with *Hin*fI or *Hae*III, electrophoresed through a 1.5% agarose gel, and 33-repeat related sequences localized by Southern blot hybridization with pAV33.7 DNA (Fig. 2). Each recombinant gave a single positive *Hin*fI and *Hae*III fragment, except for λ33.4 and 11 which gave no detectable positive *Hae*III fragments (because of the presence of a *Hae*III cleavage site in the repeat regions in these clones; data not shown). Suitable positive *Hin*fI and *Hae*III fragments were isolated by preparative gel electrophoresis[33], end-repaired if necessary and blunt-end ligated into the *Sma*I site of M13mp8 (ref. 37). M13 recombinants were isolated after transformation into *E. coli* JM101 and sequenced by the dideoxynucleotide chain-termination method[38,39]. Each subcloned λ33 fragment contained a tandem repetitive region which in some cases could be sequenced directly. In other cases where the repeat region was too far from the sequencing primer site, the M13 inserts were shortened by cleavage with suitable restriction endonucleases and resequenced.

estimated at 1.0×10^{-9} substitution per nucleotide site per year[25–27]. Assuming that the generation time in man is 20 yr, this predicts a base substitution mutation rate of 2×10^{-5} per 1-kb minisatellite per gamete, lower than the estimated unequal exchange rate of 10^{-4} per gamete. This disparity in rates[23] is probably sufficient to maintain the amount of repeat sequence homogeneity seen in the hypervariable minisatellites λ33.1, 4 and 6.

The rate of unequal exchange can therefore be as high as 10^{-4} per kb minisatellite sequence and presumably is proportional to minisatellite length. In contrast, the rate of homologous recombination at meiosis in human DNA is ~1 centimorgan per 10^6 bp (ref. 2) or 10^{-5} per kb. The apparently very high rate of unequal exchange in minisatellites suggests either that they are hotspots for meiotic recombination, or that most exchanges are between sister chromatids at mitosis in the germline.

A χ sequence in minisatellites?

The length and sequence of the consensus minisatellite repeat sequences vary considerably; none of them are flanked by direct repeats (Fig. 3), in contrast to the repeat region in the myoglobin gene (Fig. 1). Thus, it is unlikely that these minisatellites are related by transposition of a common ancestral sequence. We therefore used dot-matrix comparisons[28] of each minisatellite repeat consensus with the myoglobin 33-bp repeat sequence to determine which region(s) of the 33-bp repeat probe were detecting each minisatellite. Remarkably, the consensus sequence of each minisatellite repeat aligns with the myoglobin repeat specifically over a unique 10–15-bp core region of the 33-bp probe sequence (Fig. 4). This shared core region consists of an almost invariant sequence GGGCAGGAXG preceded by a 5-bp sequence common to most, but not all, repeats.

This core region in each cloned minisatellite suggests strongly that this sequence might help to generate minisatellites by promoting the initial tandem duplication of unique sequence DNA and/or by stimulating the subsequent unequal exchanges required to amplify the duplication into a minisatellite. As polymorphic minisatellites may also be recombination hotspots (see above), it might be significant that the core sequence is similar in length and in G content to the χ sequence, a signal for generalized recombination in *E. coli*[29] (Fig. 4*a*). Although the precise function of χ is unknown, current recombination models[30] suggest that this sequence binds the *rec*BC gene product, endonuclease V, which unwinds locally and nicks DNA to produce a single-stranded DNA projection required for the generation of Holliday junctions. In principle, DNA repair synthesis from the nicking site, followed by ligation to the single-stranded DNA projection, could generate a short tandem duplication with each duplicate containing a χ sequence capable of promoting unequal exchange and amplification of the duplicated region to produce a minisatellite (Fig. 4*b*). Although this model is highly speculative, it predicts that isolated core (or core-like) sequences may also be hotspots for initiating human chromosome recombination.

Probe for hypervariable regions

The repeat length of each minisatellite region is usually half (λ33.5 and 15), the same (λ33.3, 6, 10 and 11) or double the length (λ33.1 and 4) of the 33-bp probe from the human myoglobin gene (Fig. 3). This suggests that detection of minisatellites by pAV33.7 depends not only on the presence of a core sequence in each repeat but also on an in-phase alignment of cross-hybridizable core sequences in a heteroduplex between a minisatellite and the 33-bp repeat probe. If this is correct, then a probe consisting only of a tandem-repeated core sequence should be able to detect not only the human DNA fragments detectable by pAV33.7, but also additional minisatellites incapable of forming stable heteroduplexes with the 33-bp repeat probe. We therefore used the minisatellite from λ33.15, comprising 29 almost identical repeats of an almost perfect 16-bp core sequence, as a hybridization probe for additional minisatellites.

As shown in Fig. 5, this repeated core probe detects a complex profile of hybridizing fragments in human DNA digested with *Hin*fI, including most, but not all, of the bands detected previously with the 33-bp repeat probe from pAV33.7 (comparative data not shown). Only the largest (4–20 kb) *Hin*fI fragments can be resolved fully; these show extreme polymorphism to the extent that the hybridization profile provides an individual-specific DNA 'fingerprint'. Large fragment hyperpolymorphism is to be expected as, if the rate of unequal exchange is proportional to minisatellite length, then long minisatellites (~10 kb long) will have a greater unequal exchange rate ($u \sim 0.001$), raising both the heterozygosity and the number of alleles in a population.

Pedigree analysis

To establish that these large highly polymorphic fragments are stably inherited and segregate in a mendelian fashion, we analysed *Hin*fI digests of DNAs taken from an extensive Asian-Indian pedigree of Gujerati origin, including 54 individuals spanning four generations. The Southern blot/gel electrophoresis time was increased to improve the resolution of these large fragments. Typical examples of part of this pedigree are shown in Fig. 5*d*, *e*, confirming that the polymorphic variation is so great that all individuals, even in a single sibship of a first-cousin marriage (Fig. 5*e*), can be distinguished.

The families in Fig. 5*d*, *e* show that most of the large *Hin*fI fragments are transmitted from each parent to only some of the offspring, establishing that most of these fragments are present in the heterozygous state and that the heterozygosity for these large hypervariable fragments must be approaching 100%. Furthermore, inheritance is Mendelian in that these heterozygous bands are transmitted on average to 50% of the offspring; 48 clearly-resolved different heterozygous parental bands were scored in four sibships of 4–6 individuals and gave a total of 116 cases in which an offspring had inherited a given parental band, compared with 124 cases where the band had not been transmitted (data not shown). Conversely, all fragments in offspring can be traced back to one or other parent, then in turn to their parents (with one exception, see below) and therefore provide a set of stably inherited genetic markers. No band is specifically transmitted from father to son or father to daughter (Fig. 5*d*), eliminating Y and X linkage respectively and implying that these minisatellite fragments are mainly autosomal in origin. Although it is not yet known from where in the set of autosomes these DNA fragments originate, they are not derived from a single localized region of one autosome. Instead, pairs of parental fragments can be identified which segregate independently in the offspring (Fig. 5*d*). Precisely, a pair of bands AB in one parent (and absent from the other) cannot be allelic, nor linked closely in repulsion, if there is at least one AB or − − offspring; the presence of A− or −B recombinant progeny further establishes lack of tight linkage in coupling between A and B. Careful examination of the original autoradiograph of the family shown in Fig. 5*d* reveals, by these criteria, at least 10 resolvable bands in the mother, eight of which are mutually non-allelic and not linked closely. Two other bands may each be an allele of one of the eight unlinked fragments, in that only A− and −B progeny are observed in the limited number of offspring analysed, although such a small sample is insufficient to prove that such pairs of fragments are alleles of a single locus. We conclude that the core probe can give useful information simultaneously on at least several distinct unlinked hypervariable loci.

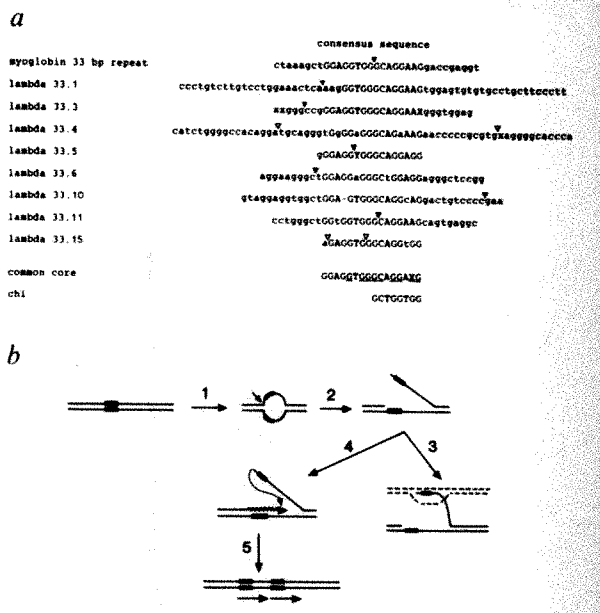


Fig. 4 A common χ-like core sequence shared by the repeat sequence of each minisatellite region. The sequence of each region was compared with the 33-bp tandem repetitive sequence in pAV33.7 and with its reverse complement, using dot-matrix analysis with variable windows and matching criteria[28]. In each case, only a single unambiguous region of sequence similarity was found between the myoglobin 33-bp repeat sequence and the λ33-repeat sequence. The same region was shared by the repeats of all eight λ33 clones. *a*, The aligned sequence of each repeat consensus, each of which is given as an arbitrary cyclic permutation of the consensus shown in Fig. 3. The common core sequence shared by all repeats is also shown and positions in each consensus which conform to the core sequence are identified by upper case letters. Invariant nucleotides in the canonical core sequence are underlined; the generalized recombination signal (χ) of *E. coli* is given also[29]. The beginning/end point of each repeat consensus (Fig. 3) is identified by ▼; in the case of λ33.4 and λ33.15, there is a non-integral number of repeats (Fig. 3) and the separate repeat beginning and end points are shown by ▽. *b*, Model for minisatellite generation promoted by χ-like sequences. (1) A χ-like region denoted by a box. RecBC enzyme binds to χ and unwinds DNA. (2) Nicking produces a single-strand projection (3), which can be assimilated into a homologous duplex to form the precursor of a Holliday junction[30]. (4) Alternatively, DNA repair synthesis followed by (5) ligation and segregation produces a tandem duplication of a χ-containing sequence which can amplify further by unequal exchange. The length of the tandem repeat is determined by the extent of repair synthesis.

A new mutant allele

The extreme variability of the large *Hin*fI DNA fragments detected by the repeat core sequence suggests that the rate of generation of new length alleles must be very high and possibly amenable to direct measurement. There is a clear instance of a new mutation in the pedigree shown in Fig. 5*e*; individual 17 has a new fragment (arrowed), not present in either parent, which might have been derived by unequal exchange and slight expansion of a smaller maternal and paternal fragment present in the other offspring. In a survey of 27 individuals and their parents (data to be presented elsewhere), 240 clearly resolved offspring bands could be traced to one or other parent, the only exception being the band in individual 17. This gives a mutation rate u to a new allele for these hypervariable fragments of

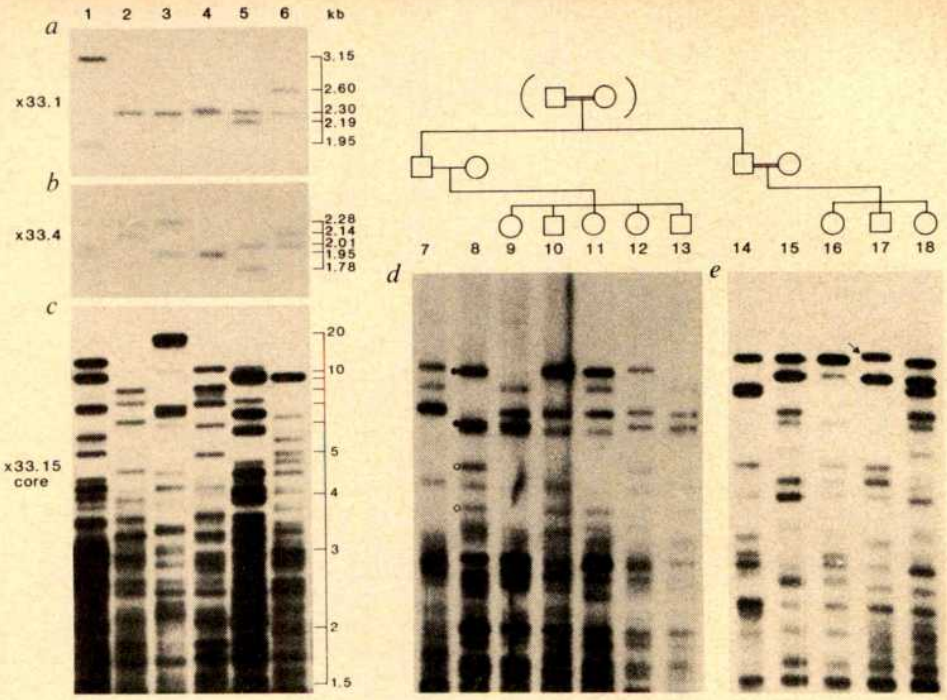


Fig. 5 Polymorphic human DNA fragments detected by hybridization with individual λ33 probes. Southern blots of *Hin*fI digests of DNA from a random sample of British caucasians (1–6) and from selected members of a large British Asian-Indian pedigree (7–18) were hybridized with single-stranded ^{32}P-labelled hybridization probes prepared from suitable M13 recombinants containing minisatellite regions. The pair of bands (○) in individual 8 is an example of non-allelic fragments which are not tightly linked; the pair marked (●) illustrates possible allelism in that each of the five offspring inherits only one of the two fragments. The arrowed fragment in individual 17 is present in neither parent and is a new mutant. The correct paternity of individual 17 has been verified as described below.

Methods. 10 μg samples of DNA prepared from white blood cells[10] were digested with *Hin*fI, electrophoresed through a 20-cm long 1% agarose gel and transferred by blotting to a Sartorius nitrocellulose filter. Single-stranded ^{32}P-labelled hybridization probes were prepared from M13 recombinants as follows. Approximately 0.4 μg M13 single-stranded DNA was annealed with 4 ng 17-mer sequencing primer[40] in 10 μl 10 mM $MgCl_2$, 10 mM Tris-HCl (*p*H 8.0) at 60 °C for 30 min. Primer extension was performed by adding 16 μl 80 μM dATP, 80 μM dGTP 80 μM TTP, 10 mM Tris-HCl (*p*H 8.0), 0.1 mM EDTA plus 3 μl (30 μCi)[α-^{32}P]dCTP (3,000 Ci mmol^{-1}) and 1 μl 5 units μl^{-1} Klenow fragment (Boehringer) and incubating at 37 °C for 15 min. Extension was completed by adding 2.5 μl 0.5 mM dCTP and chasing at 37 °C for a further 15 min. The DNA was cleaved at a suitable restriction endonuclease site either in the insert or in the M13 polylinker distal to the insert, denatured by adding 1/10 vol. 1.5 M NaOH, 0.1 M EDTA, and the ^{32}P-labelled single-stranded DNA fragment extending from the primer was recovered by electrophoresis through a 1.5% low melting point agarose gel (Sea Plaque). The excised band (specific activity > 10^9 c.p.m. μg^{-1} DNA) was melted at 100° C in the presence of 1 mg alkali-sheared carrier human placental DNA (sheared in 0.3 M NaOH, 20 mM EDTA at 100° C for 5 min) and added directly to a pre-warmed hybridization chamber; the carrier DNA also suppressed any subsequent hybridization to repetitive DNA sequences. The precise probes used were: 33.1, a 2,000-nucleotide subcloned *Hae*III fragment containing the minisatellite plus 350-nucleotide flanking human DNA; 33.4, a 695-nucleotide non-minisatellite *Eco*RI fragment on the primer-proximal side of the minisatellite contained in a 2,015-nucleotide *Hin*fI fragment; 33.15 core, a 592-nucleotide subcloned fragment containing the minisatellite sequence plus 128-nucleotide flanking human DNA. Hybridizations were performed as described elsewhere[36], except that dextran sulphate was replaced by 6% (w/v) polyethylene glycol 6,000 (Fisons) to reduce background labelling[41]. Filters A and B were hybridized overnight in 0.5 × SSC at 65 °C and washed in 0.2 × SSC at 65 °C. Filters C–E were hybridized and washed in 1 × SSC at 65 °C. Filters were autoradiographed for 1–3 days at −80 °C using a fast tungstate intensifying screen. The correct paternity of individual 17 was established using a range of biochemical and blood group markers (haptoglobin, transferrin, red cell acid phosphatase, phosphoglucomutase I, adenylate kinase, adenosine deaminase, α_1-antitrypsin, G_c, Gm, esterase D, glyoxylase, phosphoglycollate phosphatase, C3, peptidase D, ABO, Rh and HLA; S.L.T., unpublished data), and confirmed further by rehybridizing this blot with the core minisatellite in λ33.6 to generate a second DNA 'fingerprint' in which all polymorphic bands in individual 17 could be traced back to one or other parent (data not shown).

$\sim\frac{1}{240} = 0.004$. This estimate is in reasonable agreement with the population genetic estimate of $u \sim 0.001$ for these very large hypervariable fragments (see above).

Conclusions

Here, we show not only that it is possible to design probes for the cloning of individual polymorphic minisatellite regions from human DNA, but also that the shared core sequence, which possibly serves as a recombination signal and promotes the formation of minisatellites, can be used for the simultaneous analysis of multiple hypervariable regions. We anticipate that these DNA 'fingerprints' will be of general use in human segregation analysis, in particular for detecting specific bands in close linkage with disease loci in large pedigrees and for studying marker loss in tumours. In addition, they provide a powerful method for paternity and maternity testing, can be used in forensic applications and might also be useful in detecting inbreeding between couples who have had an affected offspring possibly caused by an autosomal recessive gene carried by both parents.

The precise sequence of the core consensus shared by the repeat elements of the cloned minisatellites will be biased by the particular version of the core present in the myoglobin gene minisatellite used as the initial hybridization probe. Therefore, other variant $(core)_n$ probes might detect additional polymorphic loci not found by the λ33.15 repeated core sequence. Preliminary experiments have indeed shown that the core minisatellites in λ33.5 and λ33.6 also hybridize to multiple hypervariable loci, many of which are novel. We are attempting currently to clone large hypervariable regions to provide locus-specific probes for individual minisatellites.

The detection of new mutant-length alleles of minisatellites in human pedigrees will help the analysis of rates and mechanisms of unequal exchange and gene homogenization and can in principle be used to determine whether such exchanges occur by sister chromatid exchange or by recombination between homologous chromosomes at meiosis. In addition, if the core sequence is indeed a recombination signal, then its accurate definition could provide useful substrates for studying mechanisms of human recombination.

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1 Weatherall, D J *The New Genetics and Clinical Practice* (Nuffield Provincial Hospitals Trust, London, 1982)
2 Botstein, D, White, R L, Skolnick, M & Davis, R *Am. J hum Genet* **32**, 314-331 (1980)
3 Drayna, D *et al Proc. natn Acad Sci USA.* **81**, 2836-2839 (1984)
4 Murray, J M *et al Nature* **300**, 69-71 (1982)
5 Gusella, J F *et al Nature* **306**, 234-238 (1983)
6 Cavanee, W K *et al Nature* **305**, 779-784 (1983)
7 Fearson, E R, Vogelstein, B & Feinberg, A P *Nature* **309**, 176-178 (1984)
8 Koufos, A *et al Nature* **309**, 170-172 (1984)
9 Orkin, S H, Goldman, D S & Sallan, S E *Nature* **309**, 172-174 (1984).
10. Jeffreys, A J *Cell* **18**, 1-10 (1979)
11 Cooper, D N & Schmidtke, J. *Hum Genet* **66**, 1-16 (1984)
12 Ewens, W J, Spielman, R S & Harris, H *Proc natn Acad Sci USA* **78**, 3748-3750 (1981)
13 Barker, D, Schafer, M & White, R *Cell* **36**, 131-138 (1984)
14 Wyman, A & White, R *Proc. natn Acad Sci USA.* **77**, 6754-6758 (1980)
15 Bell, G I, Selby, M J & Rutter, W J *Nature* **295**, 31-35 (1982)
16 Higgs, D R, Goodbourn, S E Y, Wainscoat, J S, Clegg, J.B & Weatherall, D J *Nucleic Acids Res* **9**, 4213-4224 (1981)
17 Proudfoot, N J, Gil, A & Maniatis, T *Cell* **31**, 553-563 (1982)
18 Goodbourn, S E Y, Higgs, D R, Clegg, J B & Weatherall, D J *Proc. natn. Acad Sci USA.* **80**, 5022-5026 (1983)
19 Capon, D J, Chen, E Y, Levinson, A D, Seeburg, P H & Goeddel, D V *Nature* **302**, 33-37 (1983)
20 Weller, P, Jeffreys, A J, Wilson, V & Blanchetot, A *EMBO J* **3**, 439-446 (1984)
21 Vieira, J. & Messing, J *Gene* **19**, 259-268 (1982)
22 Loenen, W A M & Brammar, W J *Gene* **20**, 249-259 (1980)
23 Dover, G *Nature* **299**, 111-117 (1982)
24 Morton, N E *Outline of Genetic Epidemiology* (Karger, Basel, 1982)
25 Chang, L-Y. E. & Slightom, J L. *J. molec. Biol.* (in the press)
26 Goodman, M, Koop, B F, Czelusniak, J, Weiss, M & Slightom, J L *J. molec. Biol.* (in the press)
27. Harris, S, Barrie, P A, Weiss, M L & Jeffreys, A J *J. molec. Biol* (in the press).
28 White, C T, Hardies, S C, Hutchison, C A & Edgell, M H *Nucleic Acids Res* **12**, 751-766 (1984)
29 Smith, G R, Kunes, S M, Schultz, D W, Taylor, A & Triman, K L *Cell* **24**, 429-436 (1981)
30 Smith, G. R *Cell* **34**, 709-710 (1983)
31 Ewens, W J *Theor Pop. Biol.* **3**, 87-112 (1972)
32 Ewens, W J *Mathematical Population Genetics* (Springer, Berlin, 1979)
33 Dretzen, G, Bellard, M., Sassone-Corri, P & Chambon, P. *Analyt Biochem* **112**, 295-298 (1981)
34 Maxam, A M & Gilbert, W *Meth Enzym* **65**, 499-560 (1980).
35. Southern, E M *Meth Enzym* **68**, 152-176 (1980)
36 Jeffreys, A J *et al Cell* **21**, 555-564 (1980)
37 Messing, J. & Vieira, J. *Gene* **19**, 269-276 (1982)
38 Sanger, F, Nicklen, S & Coulson, A R *Proc. natn Acad. Sci. U.S A* **74**, 5463-5467 (1977)
39. Biggin, M D, Gibson, T J. & Hong, H F *Proc. natn Acad. Sci. U.S A.* **80**, 3963-3965 (1983).
40 Duckworth, M L *et al. Nucleic Acids Res* **9**, 1691-1706 (1981)
41 Renz, M & Kurz, C *Nucleic Acids Res* **12**, 3435-3444 (1984)

LETTERS TO NATURE

Cometary impacts, molecular clouds, and the motion of the Sun perpendicular to the galactic plane

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Raup and Sepkoski[1] have presented evidence from marine fossils for a 26-Myr periodicity in the occurrence of mass extinctions. Using the same data Rampino and Stothers[2] obtained a different period, 30 ± 1 Myr, which agrees with the 33 ± 3-Myr half-period for the vertical oscillation of the Solar System about the plane of the galaxy[3]. To explain this agreement they suggest[2] that encounters with molecular clouds perturb the Sun's family of comets, causing many to enter the inner Solar System where one or more collide with the Earth; the cloud encounter rate is modulated at twice the oscillation frequency, because the number density of clouds peaks at the galactic plane at the midpoint of the solar oscillation crossed by the Solar System twice per period. Notwithstanding an apparent objection to this that the most recent extinctions are not in phase with the solar oscillation[4], their model, given its stochastic nature, can accommodate a few events with large phase discrepancies. The degree of modulation is crucial: it depends on the scale height of the population of molecular clouds relative to the amplitude of the solar motion and tends to zero if this ratio is large and encounters are entirely random. Here we present data from CO surveys of molecular clouds both within and beyond the solar circle, which permit explicit calculation of the strength of the modulation. The cloud layer near the Sun is too extended and, as a consequence, the modulation of cloud encounters is too weak for a statistically significant period to be extracted from the nine extinctions analysed by Rampino and Stothers.

Although we assume a gaussian distribution of clouds with z, the distance from the galactic plane, our conclusions do not depend sensitively on this assumption and remain essentially unchanged if an exponential or other plausible distribution function is assumed instead. The empirical parameter crucial to the mechanism of Rampino and Stothers is, then, the ratio of the amplitude of the solar oscillation z_0 to the half-thickness at half-density $z_{1/2}$ of the distribution of local molecular clouds. One is not free to treat z_0 as a free parameter; it is fixed by (1) the requirement that the period, T, of the oscillation must be 60 Myr, twice the putative extinction period, (2) the circumstance that the Sun is now close to the galactic plane so its z component of velocity, $v_\perp = 7.4\ \mathrm{km\ s^{-1}}$, is essentially that at $z = 0$, and (3) the constraint of simple harmonic oscillation[5], $z_0 = v_\perp T/2\pi = 72$ pc.

Because of the large scatter and uncertainty in the distance of local clouds, there is, as yet, no reliable measurement of $z_{1/2}$ in the vicinity of the sun (that is, within ~1 kpc) from CO or other molecular cloud surveys, but there are good measurements from several large-scale CO surveys of $z_{1/2}$ as a function of galactocentric distance for distant clouds both within the solar circle and beyond. Figure 1 summarizes the survey data. All the CO surveys are consistent with a gradual increase of $z_{1/2}$ with R, proportional approximately to $R^{0.5}$ and a value of $z_{1/2}$ at the solar circle of 85 ± 20 pc (uncertainty 1 s.d.) which we will adopt here for the solar vicinity. Local observations of clouds at known distances are consistent with this value, but are inconsistent with a population of clouds significantly more compressed to the galactic plane. The well-known large concentrations of molecular clouds in Orion and Monoceros[6], for example, representing a significant fraction of local clouds by mass, lie 150-200 pc from the plane, or about twice our adopted $z_{1/2}$.

To calculate the encounter rate as the solar system oscillates sinusoidally through a gaussian distribution of clouds, we may safely neglect the small present displacement of the Sun from the galactic plane. The solar velocity components parallel and perpendicular to the plane are then $v_\parallel = v\cos b = 18.5\ \mathrm{km\ s^{-1}}$ and $v_\perp = v \sin b \sin \omega t = 7.4\ \mathrm{km\ s^{-1}} \sin \omega t$, where $v = 20\ \mathrm{km\ s^{-1}}$ is the present solar motion relative to local stars and interstellar matter[7], $b = 22°$, the galactic latitude of the solar apex[7], and $\omega_0 = 2\pi/60\ \mathrm{Myr^{-1}}$. Because of the low latitude of the apex, the solar speed as a function of time, $v(t) = v_\parallel(1 + 0.164\cos^2\omega_0 t)^{1/2} \approx v_\parallel(1 + 0.082\cos^2\omega_0 t)$, is always large with respect to the random motion of the clouds, which may be neglected. Letting $\zeta = \ln 2\ z_0^2/z_{1/2}^2$, the number of encounters (extinctions) per unit time is then simply

$$r(t) = n(z(t))v(t)\sigma = C(1 + 0.082\cos^2\omega_0 t)\exp(-\zeta\sin^2\omega_0 t) \quad (1)$$

an explicit function of time at a given ζ with no free parameters except the constant of normalization $C \equiv n_0 v_\parallel \sigma$, where n_0 is the in-plane number density of clouds and σ the encounter cross section. It is tacitly assumed that the encounters are short range, that is, that the impact parameter b is small with respect to $z_{1/2}$; the effect of long range encounters is clearly to reduce the modulation (see below).

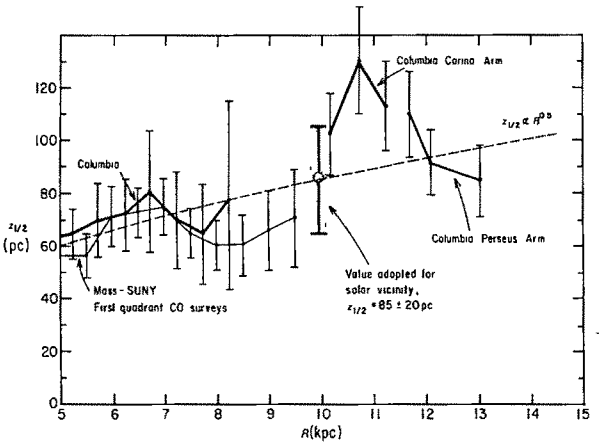


Fig. 1 Half-thickness of the distribution of molecular clouds with distance from the galactic plane, as a function of distance from the centre of the Galaxy, determined by the Massachusetts-Stony Brook (ref. 11) and Columbia CO surveys.

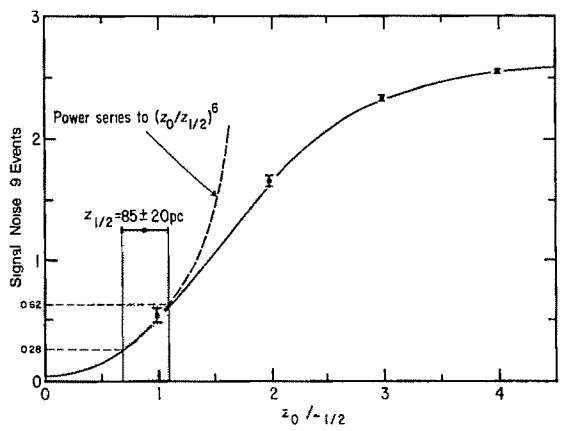


Fig. 2 Signal-to-noise of the leading Fourier component at $2\omega_0$ for nine events, as a function of the ratio of the amplitude of the solar oscillation to the half-thickness of the molecular cloud disk. The four points with vertical error bars indicate the results of a check on equation (4) with the Monte-Carlo code used to produce Fig. 3.

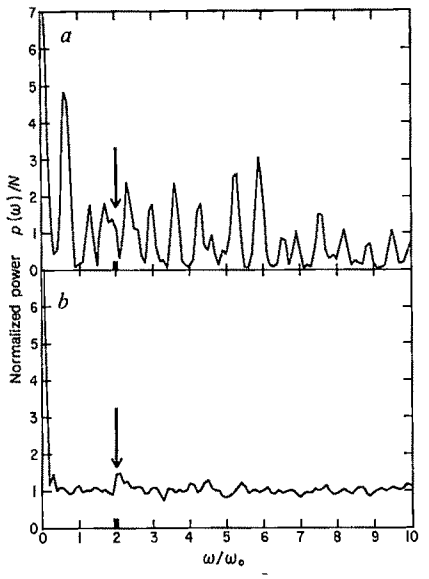


Fig. 3 Monte-Carlo calculation for $z_0/z_{1/2}=1$ of the power spectrum of random events drawn from the distribution given by equation (1). *a*, Nine events, the number of mass extinctions analysed by Rampino and Stothers[2]; *b*, the average of 100 such spectra, showing the small expectation value $\langle p(R)\rangle - \langle p(0)\rangle$ of the nine-event peak (short vertical bar at $\omega/\omega_0=2$).

We now wish to calculate the power spectrum $p(\omega)$ for a sample of N encounters drawn from this distribution with respect to time, and, specifically, the signal strength of the leading Fourier component at $2\omega_0$ relative to the shot noise, that is, the purely statistical fluctuations in the absence of modulation. We define the compex Fourier amplitude as

$$A(\omega)=\sum_{i=1}^{N}\exp(j\omega t_i)\equiv\sum_{i=1}^{N}(x_i+jy_i) \qquad (2)$$

where the t_i are the encounter times and define the power spectrum as usual as $p(\omega)=|A(\omega)|^2$. The first and second moments of $A(2\omega_0)$ are readily calculated. We may take $\langle y\rangle=0$ without loss of generality. Then, $\langle x\rangle=\int r(t)\cos 2\omega_0 t\,dt/\int r(t)\,dt\equiv R$, $\langle x^2\rangle=\int r(t)\cos^2 2\omega_0\,dt/\int r(t)\,dt\equiv S^2$ and $\langle y^2\rangle=1-S^2$, and the variances corresponding to the second moments are $\sigma_x^2=S^2-R^2$ and $\sigma_y^2=1-S^2$. The expectation value of the power at $2\omega_0$ is then

$$\langle p(R)\rangle=N^2\langle x\rangle^2+N\sigma_x^2+N^2\langle y\rangle^2+N\sigma_y^2=N(N-1)R^2+N \qquad (3)$$

Care is required in calculating the signal-to-noise ratio (S/N) as the statistical fluctuations in the absence of modulation (that is, signal) are distributed normally in A but exponentially in p; the expression for this ratio in the absence of knowledge of the phase of the signal is

$$\mathrm{S/N}=\left[\frac{\langle p(R)\rangle-\langle p(0)\rangle}{\langle p(0)\rangle}\right]^{1/2}=\sqrt{N-1}\,R \qquad (4)$$

The normalized Fourier transform R in equation (2) may be conveniently approximated analytically when ζ is not too large, by expanding the exponential in equation (1) in a power series, or it may be readily evaluated numerically at all ζ. The S/N ratio from both methods for $N=9$ events is shown in Fig. 2. For the observed thickness of the population of molecular clouds at the solar circle, S/N is evidently so small, 0.45 ± 0.17, that a statistically significant determination of the underlying modulation period cannot be obtained.

A graphic demonstration of this conclusion and a useful check as well of the analytical theory (Fig. 2), has been obtained by Monte Carlo simulation. The top of Fig. 3 shows the power spectrum derived numerically from a sample of nine random events drawn from the distribution of equation (1); the signal is clearly swamped by the purely statistical fluctuations, that is, the shot noise spikes. The expectation value of the peak power can be obtained by averaging together 100 such (independent) spectra, as in the bottom panel of Fig. 3; its smallness compared with the noise spikes in the top panel is evident. To express this result another way, scaling from equation (4) we find that even a relatively modest signal characterized by $\mathrm{S/N}=3$ would require not nine extinction events, but >300.

Given that our assumption, that the encounters between the Solar System and molecular clouds are short range with respect to $z_{1/2}$, is an oversimplification, a significantly larger number of events may actually be required. Because of the fairly flat mass spectrum of molecular clouds[8,9], the most important encounters are likely to be with large clouds or cloud complexes whose mass exceeds $1\times10^5\,M_\odot$ and these will occur mainly at impact parameters exceeding 50 pc (ref. 10). The result of such distant encounters is to increase significantly the effective thickness of the molecular cloud layer, which, by Fig. 2, further reduces the S/N ratio in the power spectrum of the peak at $2\,\omega_0$. Because of the breakdown of the impulsive approximation at large impact parameters, exactly how many additional events are required when this effect is taken into account is not clear, but we estimate that it could be a factor ≥ 10. Our conclusion is, therefore, that at least 300 mass extinctions and possibly many more, as opposed to the nine analysed by Rampino and Stothers, are required to yield statistically significant evidence for the oscillation of the Solar System about the plane of the galaxy under the molecular cloud hypothesis. It follows that if a periodicity does exist in the data, it is highly unlikely to result from encounters between the Solar System and molecular clouds.

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1 Raup, D & Sepkoski, J *Proc. natn Acad Sci. USA.* **81**, 801-805 (1984)
2 Rampino, M R & Stothers, R B *Nature* **308**, 709-712 (1984)
3 Innanen, K A, Patrick, A T & Duley, W W *Astrophys Space Sci* **57**, 511-515 (1978)
4 Davis, M, Hut, P & Muller, R A *Nature* **308**, 715-717 (1984)
5 Bahcall, J *Astrophys J* **276**, 156-168 (1984)
6 Thaddeus, P *Ann N Y Acad Sci.* **395**, 9-16 (1982)
7 Allen, C W *Astrophysical Quantities*, 252 (Athlone, London, 1973)
8 Dame, T M & Thaddeus, P *Bull Am Astr Soc* **14**, 616 (1982)
9 Casoli, F, Combes, F & Gerin, M *Astr. Astrophys* **133**, 99-109 (1984)
10 Bailey, M E *Mon Not R. Astr Soc.* **204**, 603-633 (1983)
11. Sanders, D B, Solomon, P M & Scoville, N Z *Astrophys J* **276**, 182-203 (1984)

A composite bolometer as a charged-particle spectrometer

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The measurement of radioactivity by direct conversion of nuclear radiation into a temperature rise of a calorimeter is as old as nuclear physics itself. As part of a general programme aiming at a determination of the mass of the electron neutrino, we have designed an improved version of a He-cooled composite diamond bolometer with a monolithic germanium thermistor, developed at the Laboratoire de Physique Stellaire et Planetaire (LPSP)[1]. Our approach, based on an idea by De Rujula[2], is to study the shape, near the upper end-point of the internal bremsstrahlung spectrum in electron-capture β decay. The best nucleus for a precise measurement seems to be ^{163}Ho, for which we have determined[3] the Q_{EC} value to be 2.83 ± 0.05 keV. A particularly interesting possibility is to use total absorption spectrometry[4] (calorimetry), in which the radioactive holmium forms part of the sensitive volume of the detector. With 5–6-MeV α particles impinging on the diamond wafer of the bolometer, a full-width-at-half-maximum (FWHM) of 36 keV was obtained at a temperature of 1.3 K. The theoretical resolution at 100 mK is a few electron-volts, so this new detection technique should give greatly enhanced energy resolution compared with present solid-state conductors based on charge carrier collection.

In 1903 Curie and Laborde[5] used a calorimeter to verify that the heat produced by a radioactive substance results from the absorption of its energetic radiation. After this, microcalorimetry became of great theoretical importance through the determination[6] of the 0.337-MeV average β energy of ^{210}Bi (Ra E). In fact, the contrast between this value and the maximum β-decay energy of 1.17 MeV for ^{210}Bi was one of the key arguments which led Pauli to the hypothesis of the neutrino. Microcalorimetry finally reached a sensitivity of $\sim 3\times10^{-5}$ W at 300 K[7]. It was Simon[8] who first pointed to the possibility of nuclear micro-calorimetry at low temperatures, where heat capacities are very low; Dalmazzone[9] has investigated a calorimeter at 1.8 K and reached a sensitivity of 10^{-9} W. The idea that bolometers or thermometers at low temperature could be used as detectors for radioactivity has reappeared

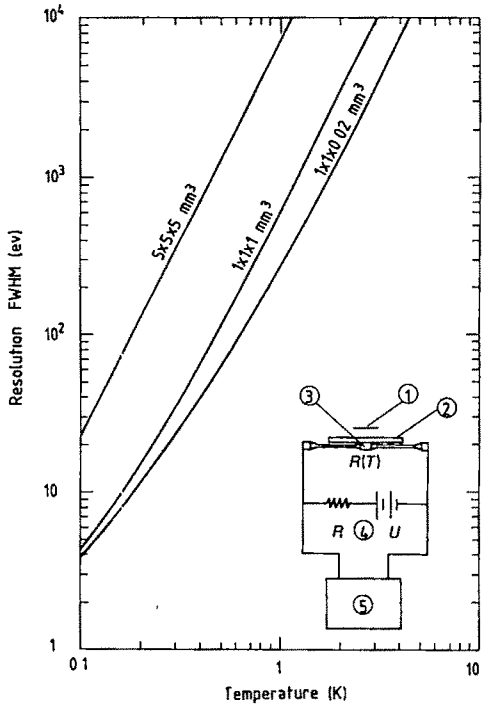


Fig. 1 Ultimate resolution with optimal filtering as a function of the temperature for the best available composite bolometer with a monolithic thermistor. The curves are calculated for three different detector volumes, V (mm^3), by means of the expression for Δv, given in the text. The 0.02-mm-thick detector represents the present technological size limit. The specific heat, C, is strongly material- and geometry-dependent and we have used the expression, $C = 7\times10^{-12}T^3 + 1.3\times10^{-12}T^{1.3} + 1.5\times10^{-12}T + 6.8\times10^{-11}VT^3$ as a good approximation to available measurements. The inset shows a schematic view of the experiment: (1) the α source; (2) the diamond substrate; (3) the Ga-doped Ge-thermistor, which also serves as mechanical support and thermal conductor to the 1.3 K thermostate; (4) the bias supply and the cooled load resistor; (5) the preamplifier–amplifier chain (at room temperature).

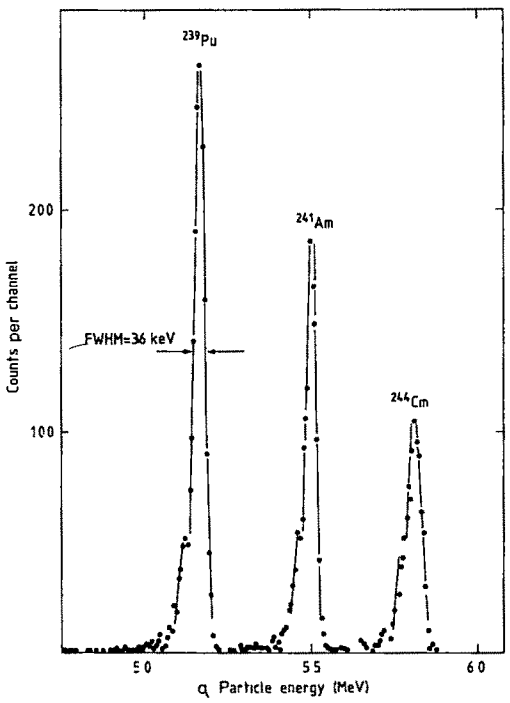


Fig. 2 Alpha energy spectrum from a mixed source of ^{239}Pu, ^{241}Am and ^{244}Cm, obtained by recording the thermal pulses induced by the α particles in a 0.25-mm^3 diamond bolometer. The resolution is 36 keV (FWHM) and the integral non-linearity is $<10^{-3}$.

recently[10-12] and it has been shown that a silicon thermistor may be used as an X-ray spectrometer[11].

The traditional use of low-temperature bolometers is for the detection of a continuous flux of infrared radiation[13-18]. The instruments based on semiconductors were found to have better performance and to be simpler to use than those based on superconductivity. The semiconductors (doped Ge or Si) have a resistance varying as T^{-4}-T^{-9} in the temperature region of 0.3-5 K. In the early 1970s a new type of bolometer, a so-called composite one, was developed at LPSP[1,14], consisting of an absorber with a large surface area (0.5-20-mm diameter) coupled thermally to a monolithic semiconductor thermistor with very small volume (typically, 0.008 mm^3). Its best performance has been obtained with a diamond wafer as the absorber and a germanium crystal as thermometer. (The use of diamond is especially advantageous because of its very high thermal diffusivity[1].) A schematic diagram of a composite bolometer is shown as an inset in Fig. 1. An asset of this construction is that there is no need for soldering, which results in a minimum of low frequency noise and a low heat capacity.

The essential advantage of the composite bolometer is that it separates the absorbing and detecting functions. The absorber (diamond, sapphire, dielectric-metal sandwich) may be optimized independently of the thermometer, which in turn has to have maximal temperature derivative of the impedance, dZ/dT, and the impedance, Z, matched to the preamplifiers[18]. The use of bolometers for detection of different kinds of flux is increasing steadily: for molecular beams[19,20], for radiation in the millimetre wave-length range[14-18] and, most recently, for X rays[11]. It is also interesting to note that spurious pulses, caused by cosmic-ray absorption in the infrared low-temperature detectors, were deliberately eliminated, using a 'spike suppression circuit', by high-altitude astronomers[21].

The diamond wafer, which acts as the absorber in our bolometer, has a volume 0.25 mm^3. At our working temperature (1.3 K) we have measured the thermalization time of the device to be <60 μs from any point of impact on its surface. The thermometer, which has a surface area of 0.1×0.1 mm^2, is glued to the diamond with a 10-μm layer of epoxy, which eliminates the possibility of charge collection, as there is no bias across the absorber.

The resolution of the detector is only limited by the noise of the detector itself (Johnson noise and thermodynamic noise) (see refs 10 and 18 for a detailed analysis of these noise effects). With optimal filtering, an order of magnitude estimate for the resolution is given by $\Delta U_{FWHM} = a\sqrt{8(\ln 2)k_B T^2 C}$ where k_B is the Boltzmann constant, T the temperature of the helium bath (in K) and C the specific heat of the bolometer at the temperature T. The constant a depends on the details of the construction of the bolometer (on dZ/dT and Z) and generally has a value of 1-2; here, $a = 1.5 \pm 0.2$. The limiting resolution calculated from this expression is shown in Fig. 1.

The bolometer used in this experiment is mounted in the vacuum chamber of a commercial portable optical cryostat[22]. A mixed ^{239}Pu, ^{241}Am, ^{244}Cm α source was positioned in front of the diamond so that no particles could reach the Ge thermometer. A Pb shutter could be inserted between source and detector during background measurements. A typical α spectrum measured at a temperature of 1.3 K is shown in Fig. 2. The low-noise preamplifier had a band pass filter of 2-120 kHz and a gain of 2,000 (ref. 23). A 5.5-MeV α particle gives a typical temperature increase of 1 mK. The resolution is 36 keV, which is not yet the best possible performance of the system. The main reason for this is that we have used standard nuclear electronics which could not be optimally matched to the time constant of the bolometer. We are presently working on improvements to both the electronics and the cryostat and hope soon to have an operational α detector with a resolution of ~3 keV at 1 K. We foresee that the technique of nuclear radiation detection via thermal pulses will have a great impact on many fields of physics.

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1 Leblanc, J, Dambier, G, Coron, N & Moalic, J P *French Patents* No.75 36103 and 75 36104 (26 Nov 1975) and *US Patent* No 4, 116, 063 26 (Sept 1978)
2 De Rújula, A *Nucl Phys* B188, 414-458 (1981)
3. Laegsgaard, E *et al* in *Proc 7th Int Conf Atomic Masses and Fundamental Constants* (AMCO-7) (ed Klepper, O) 652-658 (TDH-Schrittenreihe Wissenschaft und Technik, Darmstadt, 1984)
4 De Rújula,A & Lusignoli, M *Phys Lett* 118B, 429-434 (1982)
5 Curie, P & Laborde, A *C r hebd Séanc Acad Sci Paris* 136, 673-675 (1903)
6 Ellis, C D & Wooster, A. *Proc. R Soc.* A117, 109-123 (1927)
7 Cannon, C V & Jenks, G H *Rev Sci Instrum* 21, 236-240 (1950)
8 Simon, S *Nature* 135, 763 (1935)
9 Dalmazzone, J *Rapport* CEA-R-4858 (France), 1-39 (1977)
10 Moseley, S H, Mather, J C & McCammon, D *J appl Phys* 56(5), 1257-1262 (1984)
11 McCammon, D, Moseley, S H, Mather, J. C & Mushotzky, R F *J appl Phys* 56(5), 1263-1266 (1984)
12 Fiorini, E & Niinikoski, T *Nucl Instrum Meth* 224, 83-88 (1984)
13 Low, P J *J opt Soc Am* 51, 1300-1304 (1961)
14 Coron, N, Dambier, G & Leblanc, J in *Infrared Detector Techniques for Space Research* (eds Manno, V & Ring, J) 121-131 (Reidel, Dordrecht, 1972)
15 Clegg, P E, Ade, P A R. & Rowan-Robinson, M *Nature* 249, 530-532 (1974)
16 Roellig, T P L & Houck, J R *Int J Infrared mm Waves* 4, 299-309 (1983)
17 Lauge, A E, Kreysa, E, McBride, S E & Richards, P L *Int J Infrared mm waves* 4, 689-706 (1983)
18 Mather, J C *Appl Optics* 23, 584-588 (1984)
19 Bassi, D, Dondi, M G, Tommasini, F, Torello, F & Valbusa, U *Phys Rev* A13, 584-594 (1976)
20 Gough, T E, Miller, R E & Scoles, G *Appl Phys Lett* 30, 338-340 (1977)
21 Houck, J R & Briotta, D A Jr *Infrared Phys* 22, 215-219 (1982)
22 Dambier, G, Leblanc, J, Moalic, J P, Coron, N & Luuant, J *French Patent* No 2 410 211 (77 36217)(Nov 1977)
23 Stefanovitch, D *Rev Sci Instrum* 47, 239-240 (1976)

An apparently first-order transition between two amorphous phases of ice induced by pressure

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We recently reported[1] a transition from ice Ih to a high-density amorphous phase at 10 kbar, 77 K. Here we report that low-density amorphous ice (density 0.94 g cm^{-3}) compressed at 77 K transforms to high-density amorphous ice (1.19 g cm^{-3} at zero pressure) at a sharp transition at 6±0.5 kbar. The transition is at least as sharp as the previously reported[1] transition and strongly resembles a first-order transition in its sharpness and large volume change. It appears to be the first example of an apparently first-order transition between amorphous solids and has implications not only for our understanding of the behaviour of condensed matter, but also for theories of planetary interiors.

Ice Ih at 77 K, when compressed to its extrapolated melting pressure of 10 kbar, transforms very readily (in view of the temperature) to an amorphous solid of density 1.31± ~0.02 g cm^{-3} at 10 kbar and 1.17± ~0.02 g cm^{-3} at zero pressure[1]. The transformation seems to be of a new kind, in which a solid 'melts', at an apparently first-order transition well within the glass region of the liquid, to produce an amorphous phase that resembles the glass at the temperature and pressure of the experiment. When heated at zero pressure, the new phase transforms at ~117 K to an amorphous phase whose X-ray diffraction pattern resembles that of the amorphous ice made by condensing the vapour on a cold surface[2,3]. We have since determined the density of this phase, by weighing in liquid nitrogen, to be 0.94± ~0.02 g cm^{-3}.

The low-density amorphous ice would no doubt become less stable than the high-density amorphous ice it was made from, if it were put under high enough pressure. Eventually, it might transform to a high-density amorphous form resembling that made by squeezing ice I, perhaps at an apparently first-order transition resembling that of ice I[1] and, if so, its density would increase by ~23%. Such an apparently first-order transformation of one amorphous phase to another with a large increase in density seems not to have been reported, although gradual

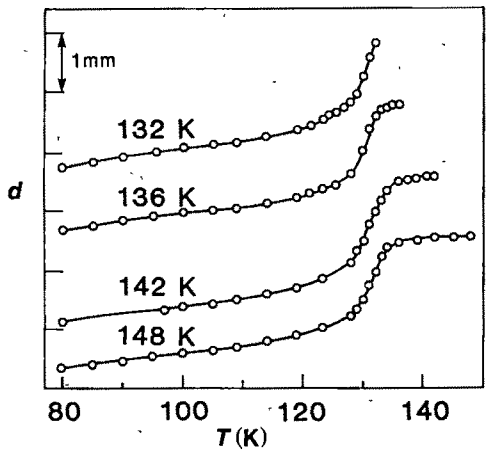


Fig. 1 The evolution of low-density amorphous ice from the high-density amorph. High-density amorphous ice was prepared by compressing about 1.0–1.2 cm^3 of water in an indium cup at 77 K to ~16 kbar in a steel vessel of 12.5 mm inside diameter, 50-mm outside diameter and 75-mm length, using a hydraulic press[1], and the pressure was reduced to a nominal pressure of 100 bar. The samples were heated in the pressure vessel at the rate of ~1 K min^{-1} to 132, 136, 142 and 148 K, as marked on the curves, and then quenched by immersing in liquid nitrogen. The temperature was measured by a thermocouple attached to the pressurizing piston and the sample may be slightly cooler than the thermocouple. The displacement of the piston is plotted against temperature in the figure.

densifications of amorphous phases of up to 20%, when squeezed to ~100 kbar with or without shearing, have been reported[4–9]. The compression of amorphous ice, reported here, should therefore be very interesting.

Four samples of high-density amorphous ice were made by compressing ice I at 77 K in an indium cup inside a steel pressure vessel[1] to ~16 kbar. The pressure was then reduced to 0.1 kbar and the pressure vessel and its contents were heated to 132, 136, 142 or 148 K at a rate of ~1 K min^{-1} to transform the high-density amorphous ice into the low-density amorph; the increase in volume was followed by the displacement of the piston. The sample was then cooled rapidly to 77 K.

The piston displacement during each of the four runs is plotted against the temperature in Fig. 1. The displacement changes rapidly with temperature above ~128 K because the high-density amorphous ice is transforming to low-density. The samples heated to 136, 142 and 148 K, which contained significantly different amounts of water, seem to have been completely transformed to the low-density form in the time of the experiment, but the sample heated to 132 K was incompletely transformed.

The low-density amorphous ice was then compressed and decompressed at 77 K in the same apparatus. The displacement of the piston during the compressions and decompressions is plotted in Fig. 2, where it is compared with the compression of a similar amount of ice Ih[1] (lowest curve). All the low-density amorphous samples compressed elastically until the sample pressure, corrected for friction, reached 6 ± ~0.5 kbar (the uncertainty is mainly due to friction in the apparatus), when a rather sudden (in view of the low temperature) transition started and the volume decreased by 4.0 ± ~0.3 cm^3 mol^{-1} or ~22%. Half of the compression occurred in a pressure range of ~0.5 kbar and the transformation seemed complete at 8–9 kbar. The transformation did not reverse when the pressure was decreased and the sample expanded only elastically. The samples that had been prepared by heating to 142 and 148 K transformed at the 6-kbar transition, but underwent another inelastic compression starting at ~10 kbar. No doubt some ice Ih[1] or Ic was formed when the samples were heated; when the samples were compressed, the ice transformed to high-density amorphous ice at 10 kbar[1], as is the case for ice Ih.

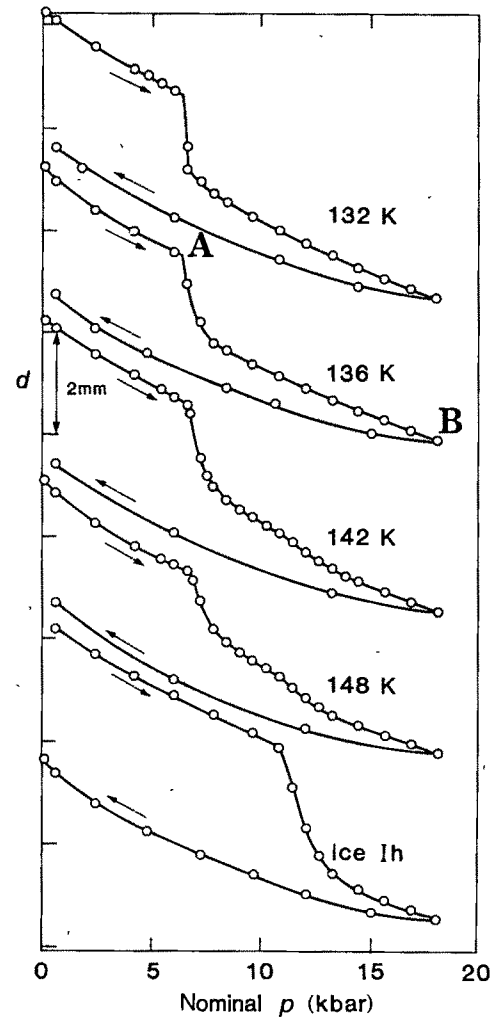


Fig. 2 Compression at 77 K to 16 kbar and decompression of the four samples of low-density amorphous ice. They were made as described in Fig. 1 legend. The arrows show the direction of the pressure changes, and the samples are identified by the temperature at which they were formed. The compression of ice Ih at 77 K is plotted as the lowest curve for comparison.

The density of the high-density phase at 6 kbar was estimated from the piston displacement to be 1.26 ± ~0.02 g cm^{-3}, and 1.19 ± ~0.02 g cm^{-3} at zero pressure. The zero-pressure density is equal, within the experimental uncertainty, to that (1.17 g cm^{-3}) of the amorph made by transforming ice Ih at 77 K and zero pressure.

Samples were recovered from the conditions represented by points A and B of the 136-K run in Fig. 2 and microphotometer traces of their X-ray diffraction patterns are reproduced in Fig. 3. The pattern of sample A is, within the experimental uncertainty, the same as that of low-density amorphous ice[1] and the pattern of sample B is the same as that of the high-density amorphous ice made by compressing ice Ih at 77 K to 10 kbar[1]. There is no doubt that the phase produced by the 6-kbar transition of low-density amorphous ice at 77 K is another amorphous phase and is similar to the high-density amorphous ice made by transforming ice I at 10 kbar[1].

The seemingly almost discontinuous transition from low-density to high-density amorphous ice with a decrease of volume of ~22% resembles, in its sharpness, the 'melting' of ice I at 77 K and 10 kbar to the 'liquid' in the glass region[1]. It seems that the low-density amorphous ice becomes unstable, relative to the high-density amorphous phase, perhaps at its surface, where ice I seems to become unstable[1], and undergoes a new kind of apparently discontinuous transition between two amorphous phases which at least approximates first-order behaviour. The phase that is produced strongly resembles the amorphous

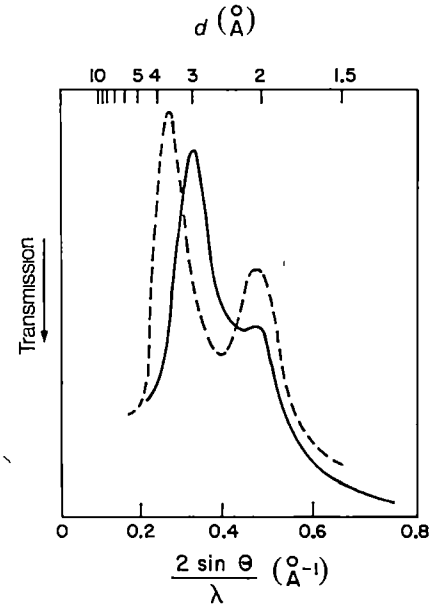


Fig. 3 Representative microphotometer tracings as a function of $(2 \sin \theta)/\lambda$, where 2θ is the scattering angle and λ, the wavelength of the X rays, of diffraction patterns of homogeneous samples of low-density amorphous ice (dashed line) and of the high-density amorphous ice (solid line) taken in a flat-plate X-ray camera. The temperature was controlled to ±5 K by flowing nitrogen gas and was measured by a thermocouple placed near the specimen. Photographs were taken at ~95 K using zirconium-filtered molybdenum radiation.

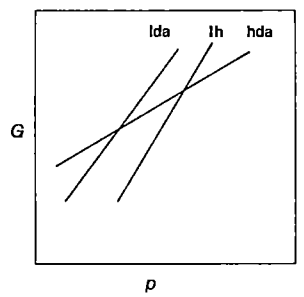


Fig. 4 Schematic graph of the Gibbs function G against the pressure p for ice Ih and low-density (lda) and high-density (hda) amorphous ice.

ice made by transforming ice I, and the transition seems to be a new way of making amorphous phases.

The pressure of the amorph–amorph transition is 4 kbar lower than that of the transition of ice I to amorphous ice at the same temperature. The difference in the transition pressures is readily understood if both transitions are considered as first-order, as they seem to be to a good approximation because they are so sharp and occur with a large volume change. The specific Gibbs functions of ice Ih, low-density amorphous ice and high-density amorphous ice are plotted schematically against the pressure in Fig. 4. From the equation $dG/dp = V$, it is readily shown that the Gibbs-function difference for two phases at zero pressure which are in equilibrium at pressure p_e is, to first order and neglecting the compressibility difference of the two phases, $\Delta G(p=0) = -\Delta V(p=0)p_e$, where ΔV is the volume difference of the two phases. The value of p_e for transformation to the high-density amorphous phase will be tentatively assumed to be the experimental transformation pressure. From the transformation pressure of ice Ih to high-density amorph[1] (hda) and of low-density (lda) to high-density amorph, and from the specific volumes of the phases at zero pressure, the Gibbs-function differences at zero pressure are $\Delta^{hda}_{Ih}G(p=0) = 4.1 \pm \sim 0.3$ and $\Delta^{hda}_{lda}G(p=0) = 2.5 \pm \sim 0.3$ kJ mol^{-1}, so that $\Delta^{lda}_{Ih}G(p=0) = 1.6 \pm \sim 0.3$ kJ mol^{-1}.

A similar transformation ought to occur in other four-coordinated amorphous solids, such as amorphous silicon and germanium. These materials crystallize when compressed at room temperature[10,11], but may form high-density amorphous phases if compressed at low temperature.

The ultimate phase of ice, as far as is known, is ice VII or VIII, both based on a body-centred-cubic arrangement of the oxygen atoms, and the corresponding phase having centrosymmetric hydrogen bonds, known as ice X[12,13]. The density of ice VIII when recovered at zero pressure and 77 K is 1.46 g cm^{-3} (ref. 14), about 25% denser than our high-density amorphous ice. The high-density amorphous ice may, therefore, undergo yet another transformation, either gradually or suddenly, if it is subjected to high enough pressures. We are presently testing this suggestion.

Finally, this amorphous–amorphous transition may be important in the planets. The ice formed in space from gaseous molecules is very probably amorphous[15] and if it agglomerates to form a planet, it will transform to the high-density amorph if the planet becomes large enough, that is, having a radius of the magnitude of 2 Mm.

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1 Mishima, O., Calvert, L. D. & Whalley, E. *Nature* **310**, 393–395 (1984)
2 Bondot, P. *C. r. hebd. Séanc. Acad. Sci., Paris* **B265**, 316–318 (1967), **B268**, 933–936 (1969)
3. Venkatesh, C. C., Rice, S. A. & Bates, J. B. *J. chem. Phys.* **63**, 1065–1071 (1975)
4 Bridgman, P. W. & Simon, I. *J. appl. Phys.* **24**, 405–413 (1953)
5 Bridgman, P. W. *Proc. Am. Acad. Arts Sci.* **84**, 111–129 (1955)
6 Cohen, H. M. & Roy, R. *J. Am. ceram. Soc.* **44**, 523–524 (1961)
7. Christiansen, E. B., Kistler, S. S. & Gogarty, W. B. *J. Am. ceram. Soc.* **45**, 172–177 (1962)
8 Cohen, H. M. & Roy, R. *Phys. Chem. Glasses* **6**, 149–161 (1965)
9 Poch, W. *Phys. Chem. Glasses* **8**, 129–131 (1967)
10 Shimomura, O. *et al. Phil. Mag.* **29**, 547–558 (1974)
11 Minomura, S., Shimomura, O., Asaumi, K., Oyanagi, H. & Takemura, K. *Proc. 7th Int. Conf. Amorphous Liquid Semiconductors* (ed. Spear, W. E.) 53–57 (Centre for Industrial Consultancy and Liaison, University of Edinburgh, 1977)
12 Hirsch, K. R. & Holzapfel, W. B. *Phys. Lett.* **101A**, 142–144 (1984)
13 Polian, A. & Grimsditch, M. S. *Phys. Rev. Lett.* **52**, 1312–1314 (1984)
14 Bertie, J. E., Calvert, L. D. & Whalley, E. *Can. J. Chem.* **42**, 1373–1378 (1964)
15 Klinger, J. *J. phys. Chem.* **87**, 4209–4214 (1983)

Al^{3+} coordination changes in liquid aluminosilicates under pressure

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Geochemists often debate the conditions in which Al^{3+} may become 6-coordinated in molten silicates and how such liquid structures are related to those of coexisting crystal phases[1–4]. To date no experimental evidence for the occurrence of Al^{3+} in this coordination state has been presented and computer simulation studies have suggested that pressures exceeding 100 kbar may be required for the 4→6 conversion[5,6]. To examine this question in the laboratory, we have taken advantage of the ability of the glass transition to freeze the structural equilibrium of a high-pressure melt and preserve it for subsequent examination in ambient conditions. Glasses of albite composition have been prepared by quenching melts under pressures of 0–80 kbar (0–8 GPa). The Al^{3+} coordination has been determined by ^{27}Al solid-state NMR spectrometry. We find that the 4-coordinated state is retained to pressures well beyond 30 kbar. A new peak at −16 p.p.m. relative to $Al(H_2O)^{3+}_6$, which we associate with octahedral Al^{3+} appears weakly at 60 kbar and becomes a prominent feature of the spectrum at 80 kbar.

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Before proceeding, we should point out that the 'fluctuations' about the average structure, which are characteristic of the liquid state, are also preserved during the high-pressure quench. This means that ambient pressure studies of the glass by small angle X-ray scattering[7], or by equivalent techniques[8], may yield another quantity much sought by geochemists, namely, the liquid silicate compressibility, κ_T. This is because frozen-in density fluctuations $\Delta\rho$, rather than composition fluctuations Δc, will in general dominate the scattering[9]. The compressibility is obtained via the well-known statistical thermodynamic relation, $\kappa_T = -\langle\Delta\rho\rangle^2 V_m/\rho^2 RT$ (ref. 10) and we hope to report such measurements soon.

For this study, albite was synthesized by heating the mixture of reagents Na_2CO_3, $SiO_2 \cdot x(H_2O)$, and $Al_2O_3 \cdot x(H_2O)$, at 1,100 °C for 20 h at 1 atm. The sample was ascertained to be a pure single phase of albite by means of X-ray diffraction. Glass samples were synthesized at various pressures of 1 atm, 20, 30, 60 and 80 kbar. The glass sample at 1 atm was prepared by heating the crystalline albite at 1,350 °C for 30 min and by quenching with a cooling rate $\sim 10^3$ deg s^{-1}.

A piston-cylinder apparatus was used to synthesize albite glass at pressures of 20 and 30 kbar. Graphite was used for the furnace material and a Pt/(Pt+10% Rh) thermocouple was used to measure the temperature. Crystalline albite, which was inserted into a Pt capsule, was heated to 1,500 °C at 20 kbar and to 1,600 °C at 30 kbar for 5 min, and glass was obtained by quenching the charge isobarically by switching off the heater power. The cooling rate was estimated to be $\sim 0.5\text{-}1 \times 10^3$ deg s^{-1}.

Glass samples at pressures of 60 and 80 kbar were synthesized using a multiple-anvil high-pressure apparatus recently constructed at the Australian National University, the details of which are given elsewhere[11]. The heater material was again graphite, and a (W+5% Re)/(W+25% Re) thermocouple was used to measure the temperature. Crystalline albite, added to graphite or boron nitride capsules, was heated above the melting temperature (1,650-1,850°C at 60 kbar, ~2,000 °C at 80 kbar) for about 1-2 min, after which the charge was quenched by cutting the power as before The cooling rate was estimated to be $\sim 1\text{-}10 \times 10^3$ deg s^{-1}. The melting periods used should be well in excess of those needed to obtain structural equilibrium in a melt formed from a single-phase solid in which only local relaxation occurs on melting. Even at the glass transition temperature T_g, the structural relaxation time (which scales with the viscosity) is ~1 min[12] and, at its melting point, the viscosity of albite is six orders of magnitude less than it is at T_g. (Some highly viscous melts nevertheless seem to retain some memory of the crystal structure from which they are derived insofar as they nucleate the parent crystal more easily than expected from long-time melting data, though we have no evidence for this in the present case.)

Despite the higher cooling rates for the high-pressure samples, it was difficult to avoid crystallization completely in the latter cases. Crystallized regions containing jadeite occurred at the sample ends where the cooling rate was smallest. These were identified under the microscope and removed before spectra were taken. One sample was deliberately crystallized to obtain a jadeite (6-coordinated Al) standard.

Because the volume of sample obtainable at 60 and 80 kbar was so small, the products of three separate preparations were combined to obtain the NMR spectra. Even so, the total mass of sample on which the 80-kbar spectrum was obtained was only 23 mg, a marginal quantity. To obtain the spectrum displayed in Fig. 2 for this sample, we had to collect and average 700,000 individual scans, requiring a total instrument time of 13 h.

NMR spectra were obtained on a Bruker CXP 300 spectrometer using the samples as synthesized (usually fragmented) without further treatment. Glass samples were suspended in powdered KBr in the Andrew type spinner. The KBr was useful for checking the spinning angle[13,14]. Samples can easily be separated from the KBr when desired by dissolving the KBr in water.

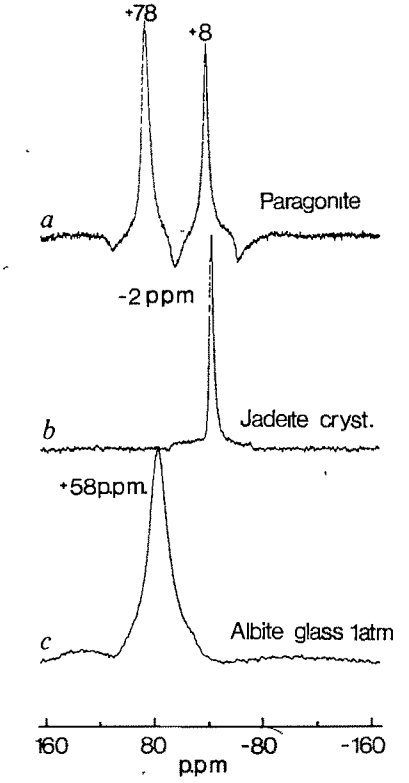


Fig. 1 ^{27}Al-NMR spectra in *a*, crystalline paragonite $(NaAl_2(Si_3AlO_{10})(OH)_2$, *b*, jadeite $(NaAlSi_2O_6)$; and *c*, albite $(NaAlSi_3O_8)$, showing positions of the resonance for Al^{3+} in tetrahedral and octahedral sites referenced to $Al(H_2O)_6^{3+}$ in aqueous solution.

The ^{27}Al spectra were recorded at 78.2 MHz in a field of 7 T in the Fourier transform mode. The sweep width was set to 30,000 Hz and memory size to 8 K. The usual $\pi/6$ pulses of 4 μs were applied and 10,000 free induction decays (FIDs) were collected with a repetition rate of 100 ms. A dead time of 200 μs was chosen to avoid any contribution from Al metal from the probe. The rotation rate was chosen to be 2,500 Hz (32 p.p.m.) to keep rotation bands outside the range of interest, but still inside the spectrum (as is well exemplified by the paragonite spectrum (see Fig. 1*a*)). Aluminium chemical shifts were measured relative to a solution of 1 M $Al(NO_3)_3$ in 1 M HNO_3.

^{27}Al chemical shifts are known to be very sensitive to the Al coordination state[14] and hence make an excellent probe for structural changes. Mostly, the ^{27}Al resonances in different coordination states fall into two distinct regions with almost no overlap. We illustrate this in Fig. 1 using aluminosilicates of known structure that are related to the structure of albite.

In paragonite, $NaAl_2(Si_3AlO_{10})(OH)_2$ (a Na-mica), 33% of the Al^{3+} occurs in tetrahedral sites, 66% in octahedral sites. The latter appear to contribute less intensely to the spectrum (because of broadening by the second order quadrupole interaction) and no quantitative information can be extracted presently from peak areas or intensities. The tetrahedral Al^{3+} resonance occurs at 78 p.p.m. and the octahedral peak at +8 p.p.m. relative to $Al(H_2O)_6^{3+}$. In crystalline jadeite $(NaAlSi_2O_6)$ (which was here prepared by melt crystallization at 60 kbar), all Al^{3+} ions are in octahedral sites, and the resonance occurs at −2 p.p.m. (Fig. 1*b*).

As Müller *et al.* have pointed out[15], the ^{27}Al chemical shifts are sensitive to the second nearest-neighbour coordination shell, the tetrahedral spectrum being the more sensitive to this effect. Thus, albite glass, with tetrahedrally coordinated Al^{3+} (Fig. 1*c*), has its resonance at 58 p.p.m., 20 p.p.m. downfield from the equivalent paragonite band. For jadeite, where Al^{2+} is octahedrally coordinated, the shift from the equivalent paragonite band is only 10 p.p.m. In each type of site in paragonite, Al^{3+} evidently experiences a higher electron density than in jadeite or albite,

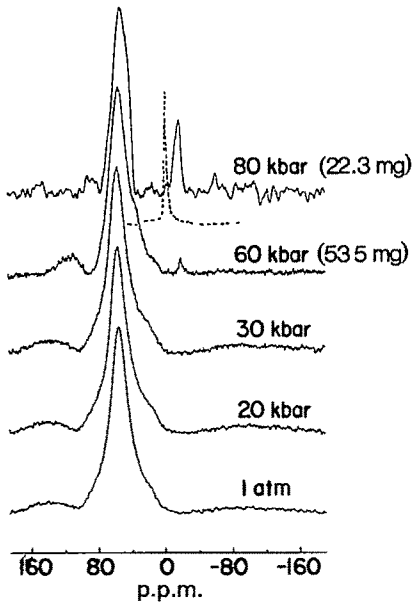


Fig. 2 ^{27}Al-NMR spectra of glassy albite samples cooled from the melt at normal pressures (lower spectrum) and at 20, 30, 60 and 80 kbar, as marked. All spectra have been normalized to the same tetrahedral peak intensity. The spectrum of ^{27}Al in jadeite (scaled to half size) is included as a dashed curve for comparisons of chemical shift with the glass octahedral component. The latter is broader than the unscaled jadeite spectrum by ~50% at half height, but somewhat narrower than octahedral Al in ambient pressure phosphate glasses[13].

which is presumably caused by a greater separation of the Al^{3+} from the between-sheet charge-compensating alkali cation in the mica. The best illustration of the spectral sensitivity to such second-neighbour effects may be seen from the study of natural feldspars containing a variety of charge-compensating cations that give rise to multiple tetrahedral site resonances, one for each type of cation, separated by ~5 p.p.m. (our unpublished results). Site distortions due to variations in local topology in a glass may be expected to produce a similar though continuous spread of resonance frequencies.

The spectra for the normal and high-pressure albite glasses are collected in Fig. 2. Because of its importance in interpreting the 80-kbar glass sample spectrum, the jadeite spectrum is also included in Fig. 2 (light-dashed peak). It is clear that no changes in the coordination or even the site symmetry of Al^{3+} in albite glass occur under pressure increases of up to 30 kbar at least. Indeed, the spectra, once normalized to the same peak intensity, can be superimposed almost within the noise level. The persistence of this spectrum over such a pressure range implies that octahedrally coordinated Al^{3+} ions are probably not to be found in any ambient pressure oxide glasses[4] unless the oxide ions being coordinated are pre-polarized by powerful species such as P(V)[13] S(VI) or Re(VII). Our results are also consistent with the absence of evidence for octahedral Al^{3+} in an earlier Raman spectral study of jadeite glass vitrified under pressures up to 40 kbar.[3] In the absence of coordination changes, the compression of liquid albite must be accomplished by decreases in the Al–O–Si and Si–O–Si bond angles. The compression involves rotation of the tetrahedral packing units to permit closer oxygen packing, as described earlier for the liquid state compaction of SiO_2 itself[16]. This will occur more easily for adjacent AlO_4 tetrahedra and SiO_4/AlO_4 pairs than for SiO_4/SiO_4 pairs because of the smaller cation–cation repulsion involved. It seems consistent with such effects that the diffusion coefficients of trivalent cations should rise more rapidly with compression than those of tetravalent cations, as shown by Kushiro's elegant interdiffusion experiments on jadeite-type couples[17].

Unfortunately, we do not have samples in the range 30–60 kbar. It seems that somewhere in this range the tetrahedral AlO_4 units have begun to suffer some distortion (note the shoulder on the 60-kbar spectrum), but the factors governing the line shape in ^{27}Al-NMR are sufficiently complex that we are presently unable to specify the nature of the distortion. ('Fingerprinting' with crystalline samples of known tetrahedral distortions and also with 5-coordinated Al^{3+} will be described elsewhere.) Together with the increased distortion is the simultaneous appearance of a small peak of −16 p.p.m., which rises above the noise level in the 60-kbar spectrum. In the 80-kbar sample spectrum, this new band has risen to almost half the height of the residual tetrahedral spectrum. Comparison with the paragonite spectrum suggests that a considerable fraction of the Al^{3+} is now in octahedral coordination. In the 80-kbar spectrum the tetrahedral spectrum is narrower and indeed its full width at half height is somewhat smaller than in the glass prepared in ambient conditions.

The new band at −16 p.p.m. is evidently not attributable to residual unseparated jadeite, the spectrum of which peaks at −2 p.p.m. (see Fig. 2). Only jadeite and co-crystallized coesite were detected in the X-ray examinations (and removed, see above), so the new band seems unlikely to be attributable to crystalline material, despite its narrowness. The possibility that it is a metastable jadeite precursor phase distributed in crystallites too small (<100 Å) to give detectable X-ray lines, although unlikely, cannot be eliminated without performing annealing studies on the high-pressure glass. These will be performed once the glass has been fully characterized. Al_2O_3, an alternative possible phase in high-pressure crystallization conditions, has a resonance in the range +4–0 p.p.m. according to our examination of samples from eight different sources, and hence cannot explain our results.

The simplest interpretation of our observations is that the initial effect of very high pressure ($P > 40$ kbar) on the framework liquid is a distortion of the tetrahedral symmetry around Al^{3+} (note the shoulder, Fig. 2), which is then relieved at higher pressure ($P > 60$ kbar) by the splitting off of a tightly defined subgroup of octahedrally coordinated Al^{3+} ions, the concentration of which will depend on the applied pressure.

These observations are not inconsistent with the results of ion dynamics computer simulation studies on liquid jadeite[5,6], which showed that both Al and Si coordination numbers could be changed with application of sufficient pressure to the high-temperature liquid. The change occurred at lower pressures for Al^{3+}, ('midpoint' at about 100 kbar) than for Si (midpoint at ~200 kbar). However, because of the high temperature that had to be maintained in these computations (for structural equilibrium to be reached within acceptable computation times), distinct coordination states were not discernible; the midpoint corresponded to an average coordination of five.

The observations are also qualitatively consistent with predictions based on the assumption of structural similarities between melt and the crystalline phase with which it is in equilibrium (quasi-crystalline melt model). For instance, jadeite only occurs at the albite liquidus above 32 kbar pressure at ~1,430 °C[18], so both 20- and 30-kbar samples, in which Al is exclusively in 4-coordination (Fig. 2), would have albite on the liquidus. The $6 \rightarrow 4$ equilibrium is presumably dependent on temperature, and it should be remembered that the equilibrium being frozen is that which exists at the glass transition temperature, T_g, which usually is at 0.6–0.7 T_m. Because the albite/jadeite boundary on the T/P phase diagram has a positive slope, the quasi-crystalline model evidently overestimates the tendency towards octahedral coordination of Al in the melt. It suggests, however, that the 6-coordinated state of Al is more easily generated at the jadeite composition although a poorer resistance to crystallization, probable at this composition, could hamper its observation by the present 'preserve-and-look' approach.

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1 Waff, H S *Geophys J Res Lett* **2**, 193-196 (1975)
2 Velde, B & Kushiro, I *Yb Carnegie Instn Wash* **75**, 618-620 (1975)
3 Sharma, S K, Virgo, D & Kuchiro, I *Am Miner* **64**, 779-787 (1979), *J. non-cryst Solids* **33**, 235-248 (1979)
4 De Jong, B H W S, Schramm, C M & Paziale, V E *Geochim cosmochim. Acta* **47**, 1223-1236 (1983)
5 Angell, C A Cheeseman, P A & Tamaddon, S *Science* **218**, 885-887 (1982)
6 Angell, C A, Cheeseman, P A & Tamaddon, S *Bull Miner* **1-2**, 87-99 (1983)
7 Renniger, A L & Uhlmann, D R *J non-cryst Solids* **16**, 325-327 (1974)
8 Buccaro, J A & Dardy, H D *J non-cryst Solids* **20**, 149-151 (1976)
9 Laberge, N. L Vasilescu, V V, Montrose, C J & Macedo, P B *J Am. ceram. Soc* **56**, 506-509 (1973)
10 Landau, L & Lifschitz, E M *Statistical Physics* Ch 12 (Pergamon, London, 1958)
11 Ohtani, E *J Phys Earth* **27**, 189-208 (1979)
12 Barkatt, R & Angell, C A *J chem Phys* **70**, 901-911 (1979)
13 Müller, D *Phys Chem Glasses* **24**, 37-42 (1983)
14 Muller, D, Gessner, W, Behrens, H J & Scheler, G *Chem Phys Lett* **79**, 59-62 (1981).
15 Muller, D, Hoebbel, D & Gessner, W *Chem Phys Lett* **84**, 25-28 (1981)
16 Woodcock, L V, Angell, C. A & Cheeseman, P A *J chem Phys* **65**, 1565-1577 (1976)
17 Kushiro, I *Geochim cosmochim. Acta* **47**, 1415-1422 (1983)
18 Bell, P M & Roseboom, E H *Miner Soc. Am. spec. Pap* **2**, 151-161 (1969)

ESR dating of volcanic ash

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Radiation-induced centres of Al, Ti, Ge and E′ occurring in the natural quartz of various geological materials were discussed a decade ago by McMorris[1,2]; but it is only recently that the peroxy centre for natural flint[3], the E′ centre for quartz from a fault[4] and the Ge centre for quartz from welded tuff[5] have been used for electron spin resonance (ESR) dating. The ages of volcanic ash or lava have been obtained by thermoluminescence (TL) dating using fine (2–11-μm) volcanic glass[6] or plagioclase feldspar[7], respectively. However, ESR dating of volcanic ash has proved extremely difficult because signal intensities of radiation-induced centres measured at room temperature are weak compared with the interfering signals from paramagnetic impurities such as Fe^{3+} and Mn^{2+}. Here we have used a signal from a quartz aluminium centre, measured at 77 K, where it is several orders of magnitude greater than those of the E′ or Ge centres at room temperature. The aluminium-centre ESR signal can also be observed in plagioclase feldspar and volcanic glass, allowing the age of the volcanic debris to be determined using any of the three mineral compounds.

The method was tested in three samples whose ages had already been obtained by other methods. Quartz was extracted from Tamagawa rhyolite welded tuff (TM1) in Hachimantai (north-east of Japan), plagioclase from Aira-Tn ash (TTS34) of Kokubu in Kagoshima Prefecture (south of Japan) and volcanic glass from volcanic ash (TE5) in the Ohiso hills (middle of Japan) or from the upper portion of the Takio Formation in the Ohita Group (south of Japan). Quartz grains, 2–3 mm in diameter, from the Tamaga welded tuff were hand picked after crushing the rock and then cleaned by 3 M HCl. The grain was crushed gently and sieved to obtain 0.25–0.125-mm particles. Volcanic glass and plagioclase feldspar were isolated by heavy liquid separation (with specific gravities of 2.5 and 2.8) after the sample has been washed in water, sieved to 0.25–0.125 mm and cleaned both ultrasonically and with 3 M HCl. Each sample was examined microscopically and found to be of greater than 90% purity. For the ESR measurement, 200-mg samples of volcanic glass and 100-mg samples of quartz and plagioclase were used. An X-band spectrometer (JEOL FE) was used at 77 K with 100-kHz field modulation. Artificial γ-irradiation was carried out with a ^{60}Co source. Measurements were repeated five times with the same sample so that average values could be obtained. The concentration of uranium and thorium was determined by spectrophotometry using arsenazo III and torin, respectively, after solvent extraction of tri-*n*-octylphosphine oxide (TOPO)/cyclohexane[8,9]. The potassium content was determined by atomic absorption spectrometry[10].

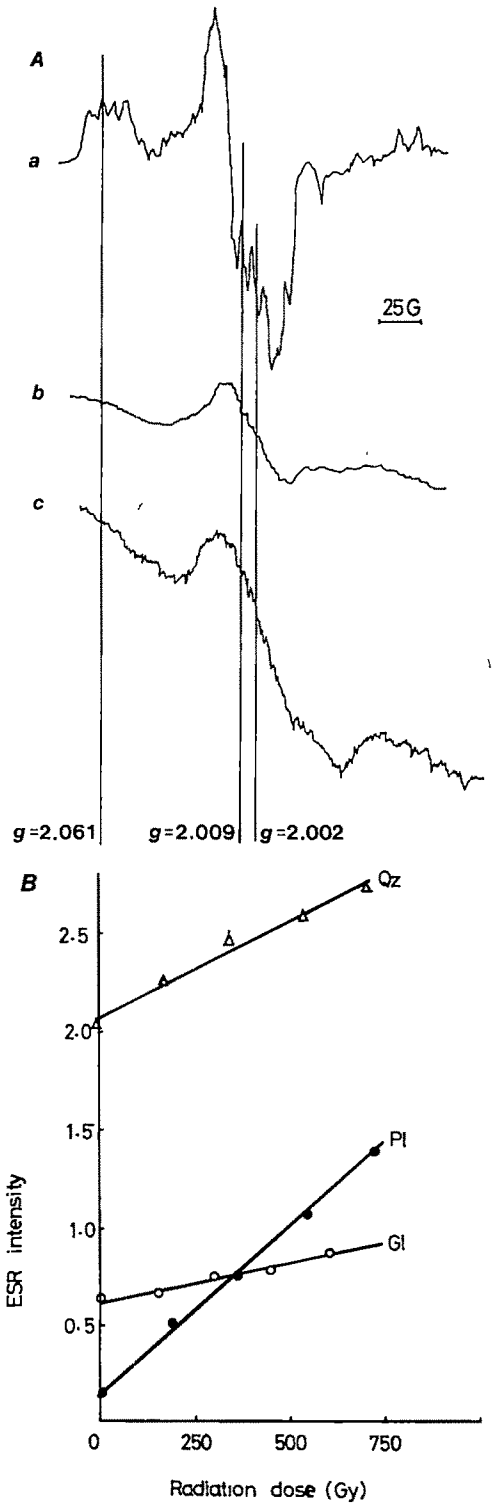


Fig. 1 *A*, The ESR spectra of Al centre in natural quartz TM1 (*a*), plagioclase TTS34 (*b*) and volcanic glass TE5 (*c*). The principal values of the *g* tensor of this centre[11] are shown. *B*, The relation of signal intensity with radiation dose for quartz (△), plagioclase (●) and volcanic glass (○).

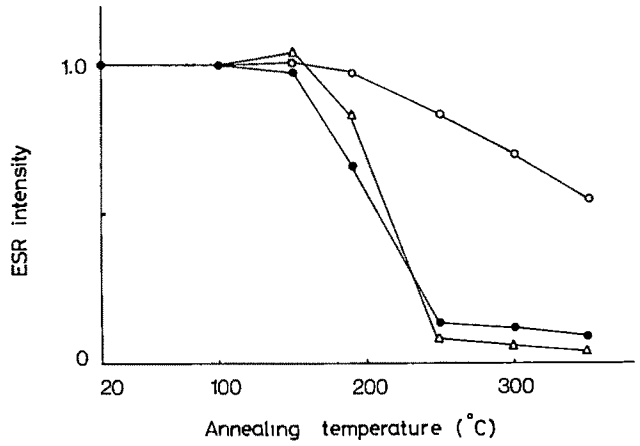


Fig. 2 Thermal annealing of Al centre in quartz TM1 (△), plagioclase TTS34 (●) and volcanic glass TE5 (○). Annealing temperature was maintained for 15 min in each step.

Figure 1*A* shows the ESR spectra of the aluminium centre in natural quartz, plagioclase and volcanic glass. An aluminium centre is a defect in which an aluminium ion replaces the silicon site and a hole is trapped at one of the four ligand oxygens. Schnadt and Räuber reported the ESR spectrum of this defect in quartz powder and fused glass[11]. The signal is measured at 77 K and broadens beyond the detection at room temperature in quartz powder. The signal intensity in plagioclase and volcanic glass is several times smaller because of extra line broadening due to a more distorted crystal field or to a looser lattice structure.

We have investigated the thermal annealing of the Al centre. Isothermal experiments were performed at several temperatures for quartz (TM1), plagioclase (TTS34) and volcanic glass (TE5) and the mean lifetimes of the traps were estimated to be 1.8–3.8 Myr, ~4 Myr and 10^3 Myr, respectively, at 10 °C. The frequency factor was assumed to be $3\times10^{12}\ s^{-1}$ and trap depth was 2.4 eV for quartz and plagioclase and 2.8 eV for volcanic glass. The changes of the ESR signal for quartz, plagioclase and volcanic glass on successive heating (for 15 min) are shown in Fig. 2. The quartz and plagioclase signals almost disappeared above 250 °C, whereas that of volcanic glass changed only slightly at that temperature, indicating that the aluminium centre in volcanic glass is much more stable. Moreover, above 250 °C, in plagioclase and volcanic glass, a large peroxy-centre signal appeared in the middle of the aluminium centre signal.

Figure 1*A* shows the ESR spectra of the aluminium centre the irradiation dose. The total dose was obtained by extrapolating the least-square-fitting line. The radiation for the minerals is assumed to be the sum of the internal α radiation and the external β and γ radiations considering the grain size of 0.25–0.125 mm. The annual dose was calculated from the analyses of the internal and bulk contents of uranium, thorium and potassium using Bell's data[12]. The α-efficiency, or k factor, is tentatively assumed to be 0 for quartz[4], 0.20 for plagioclase[7] and 0.04 for volcanic glass[13]. The internal and bulk uranium, thorium and potassium concentrations, annual dose and ESR age are shown in Table 1. For our calculation the internal α dose for TTS34 plagioclase was taken as only 6%, because the internal contents of uranium and thorium are very small, whereas that for TE5 volcanic glass is 11%. In addition to this, the external α dose (the size effect) gives an error of 7% for plagioclase and 0.6–1.4% for volcanic glass assuming a 10% external α-dose contribution[13]. However, these were not included in the calculation.

The ESR age of quartz samples (TM1) was 1.13 Myr, which agrees with the fission track age of 1.2 Myr (ref. 14). The ESR age of plagioclase of TTS34 was 2.8×10^4 yr. The ^{14}C age of widespread tephra of Aira-Tn (AT) ash has been determined to be $2.1–2.2\times10^4$ yr (ref. 15). (Note, however, that ages of $2–10\times10^4$ yr were obtained for volcanic glass samples of the AT ash from several different locations.) The ESR age of the volcanic glass of TE 5 is 0.34 Myr which agrees well with the fission track age of 0.39 Myr obtained using zircon[16]. This agreement encouraged us to apply the method to a suite of volcanic ashes of unknown ages.

The ESR ages of six volcanic ash layers in the Takio Formation of the Ohita Group were obtained using volcanic glass, as shown in Table 2. The concentration of uranium, thorium and potassium varies in each bed and, hence, the annual dose varies from 1.69 to 3.89 Gy yr^{-1}. However, the ESR age increases from 0.49 to 0.82 Myr in agreement with the stratigraphical sequence. The volcanic ash in this area has been correlated with the upper part of the Ohsaka Group (0.3–0.8 Myr)[17].

Another possible use of ESR is for 'fingerprinting' the ash layers. The ESR spectra of the aluminium centre in the AT ash from different locations have the peroxy-centre signal in the middle of aluminium-centre signal. In our work, two out of three samples of plagioclase and five out of eight samples of volcanic glass have peroxy-centre signals. In other volcanic ash, none of the 15 samples of plagioclase and only two out of 28 samples of volcanic glass exhibit this signal, which may thus be unique to AT ash. In the annealing experiment, the peroxy centre appears above 250 °C for plagioclase and volcanic glass, which suggests the mechanism by which this signal is formed.

Table 1 Radioactivity data, total dose and ESR ages for samples of known age

Mineral	U (bulk p.p.m.)	U (internal p.p.m.)	Th (bulk p.p.m.)	Th (internal p.p.m.)	K (%)	Annual dose (Gy yr^{-1})	Total dose (Gy)	ESR age (Myr)	Age from other work (Myr)
Quartz (TM)	1.2	—	4.0	—	1.48	1.96	2,210	1.13±0.22	1.2±0.3 (ref. 14)
Plagioclase (TTS34)	2.6	0.2	6.9	0.75	2.44	3.64	102	0.028±0.004	0.021–0.022 (ref. 15)
Volcanic glass (TE5)	2.2	2.0	6.7	10.9	3.85	5.10	1,680	0.33±0.05	0.39±0.08 (ref. 16)

Table 2 Total dose and ESR age of volcanic ash of upper part of Takio Formation of Ohita Group

	Depth (m)	U (bulk p.p.m.)	U (internal p.p.m.)	Th (bulk p.p.m.)	Th (internal p.p.m.)	K (%)	Annual dose (mGy yr^{-1})	Total dose (Gy)	ESR age (Myr)
1	4	0.8	1.0	2.5	6.3	2.45	2.90	1,410	0.49
2	6	1.0	0.4	4.2	4.0	1.04	1.69	840	0.50
3	7	1.8	1.0	2.3	4.4	1.16	1.94	1,050	0.54
4	15	1.3	1.6	3.3	6.7	2.05	2.82	1,870	0.66
5	30	1.2	0.8	4.9	6.0	1.40	2.24	1,620	0.73
6	35	1.3	1.2	6.4	6.7	3.02	3.89	3,150	0.82

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1 McMorris, D W *Nature* **222**, 870-781 (1969)
2 McMorris, D W. *Nature* **226**, 146-148 (1970)
3 Garrison, E G, Rowlett, R M, Cowan, D L & Holroyd, L V *Nature* **290**, 44-45 (1981).
4 Ikeya, M, Miki, T & Tanaka, K *Science* **215**, 1392-1393 (1982)
5 Shimokawa, K, Imai, N & Hirota, M *Isotope Geosci* **2**, 365-373 (1984)
6 Berger, G W & Huntley, D J *PACT* **2**, 581-592 (1983)
7 Guérin, G & Valladas, G *Nature* **286**, 697-699 (1989)
8 Emura, S *Bunseki Kagaku* **19**, 637-642 (1970)
9 Pollock, E. N *Analyt chim Acta* **88**, 399-401 (1977)
10 Terashima, S *Bull geol Surv Jap* **30**, 37-43 (1979)
11 Schnadt, R & Rauber, A *Solid State Commun* **9**, 159-161 (1971)
12 Bell, W T *Archaeometry* **21**, 243-245 (1979)
13 Hennig, G. J & Grun, R *Quat Sci Rev* **2**, 157-238 (1983)
14 Tamanyu, S & Suto, S *Bull geol Surv Jap* **29**, 159-161 (1978)
15 Machida, H & Arai, F *Quat Res* **22**, 133-148 (1983)
16 Suzuki, M & Sugihara, S *Abstr A Meet Jap Ass Quat Res*, **69** (1983)
17 Mizuno, K, Yamazaki, H & Shimokawa, K, *Abstr A. Meet Jap Ass Quat Res*, **43** (1983)

Oxygen isotope fractionation between amorphous silica and water at 34–93 °C

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The fractionation factor of oxygen isotopes between quartz and water has been extensively studied from experimental[1,2], theoretical[3,4,5] and empirical viewpoints[6] and has been widely used to estimate the isotopic composition or the temperatures of water relating to various geological processes. At lower temperatures, where hydrothermal processes, diagenesis and other processes take place, however, the fractionation factor for quartz as well as other silica minerals has not been studied thoroughly. We report here experimentally determined oxygen isotope fractionation factors between amorphous silica and water at 34–93 °C, obtained by measuring the oxygen isotope ratios of amorphous silica precipitated from geothermal waters of power plants. The relationship between the fractionation factor and temperature is similar to the extrapolated relationship of quartz observed at higher temperatures. This result is useful for the oxygen isotope study of water–rock interactions at temperatures below 200 °C.

The amorphous silica was precipitated at four points (P-1, P-2, P-3 and P-4) along the reinjection lines in the Otake and Hatchobaru geothermal power plants. Although it would have been preferable to obtain the amorphous silica at many points over a wide range of temperatures, these four points were the only ones available for this experiment. The same geothermal water from the Otake station flows at P-1 and P-4; at P-2, the geothermal waters from both stations are mixed; at P-3, the geothermal water is from the Hatchobaru station. The temperatures of flowing geothermal waters are 93, 87 and 81 °C at P-1, P-2 and P-3, respectively. The temperature at P-4 gradually decreased from 70 °C to atmospheric temperature during this study because of silica scaling in the pipeline. Samples of amorphous silica were collected at 58 and at 34 °C.

The differences in the chemical character and roughness of surface of the materials on which amorphous silicas were precipitated may influence the oxygen isotope fractionation factor between amorphous silica and water. Materials such as wire, stainless steel tubing, glass tubing, rock, wooden rod and Teflon plate were immersed in the geothermal waters at P-1 and P-4. At P-2 and P-3, only wire was used. The materials were immersed in the geothermal waters for about two weeks at P-1 and P-3, and one month at P-2 and P-4. During these periods, the geothermal waters were collected at least once in a day and their temperatures, oxygen isotope ratios, and Cl^- and total silica contents were measured. At P-2, however, these measurements were done at intervals of a week. The temperatures and oxygen isotope ratios of geothermal waters fluctuated within the errors shown in Table 1.

The *p*H, and Cl^- and total silica contents are 8.3, 1,680 p.p.m. and 525 p.p.m., respectively, at P-1, and 6.9, 2,870 p.p.m. and 730 p.p.m., respectively, at P-3. The fraction of ionic silica in the total silica was also determined. The ionic silica is determined using a conventional molybdate yellow method for an aliquot of geothermal water of which the *p*H was adjusted to ~1.2–1.5 just after collection. Most of the total silica is ionic at P-1, whereas about 90 and 55% is ionic at P-2 and P-3, respectively. This is mainly because of the differences in *p*H and the time elapsing after the geothermal water was separated from the steam[7,8]. The content of ionic silica was not measured at P-4.

The precipitated silica did not contain any crystalline materials which could be detected by X-ray diffraction, except for the samples at P-1 which contain kaolinite of < 5 wt%. From the results of chemical analysis of silica scales at the geothermal plants[9], the precipitated silica seems to have impurities of less than a few per cent. Such a small amount of contamination does not significantly affect the oxygen isotope ratio of amorphous silica.

The oxygen isotope ratios of amorphous silica and geothermal water were measured using a fluorination[10] and an H_2O–CO_2 equilibration method, respectively. The isotope ratios were expressed as $\delta^{18}O$ values relative to the SMOW standard. The $\delta^{18}O$ of silica was referred to SMOW on the basis of a fractionation factor of 1.0407 between CO_2 and H_2O (ref. 11). The $\delta^{18}O$ value of NBS-28 is +9.36‰ in this method.

Table 1 $\delta^{18}O$ values of amorphous silicas precipitated from geothermal waters on various materials and for the geothermal waters, and the fractionation factor between amorphous silica and water

Sampling point	Temperature (°C)	Date	Materials	$\delta^{18}O_{SMOW}$ (‰) Amorphous silica	$\delta^{18}O_{SMOW}$ (‰) Geothermal water	Fractionation factor (α)
P-1	92.7 ± 0.3	August 1983	Iron wire	15.2	−5 4 ± 0.1	1 0207
			Wooden rod	15.6		1.0211
			Glass tubing	15.3		1.0208
P-2	87 ± 3	February 1983	Iron wire	17.9	−5 2 ± 0.2	1 0231
P-3	80.8 ± 1	March 1983	Iron wire	20.9	−4.3 ± 0 1	1.0253
P-4	58 ± 10	December 1982	Iron wire	22.7	−5.4 ± 0.1	1.0283
			Glass tubing	23.1		1.0287
			Teflon plate	22.8		1.0284
			Rock	23.0		1.0286
			Stainless steel tubing	23.1		1.0287
P-4	34 ± 5	February 1983	Iron wire	27.5	−5.4 ± 0 1	1.0331

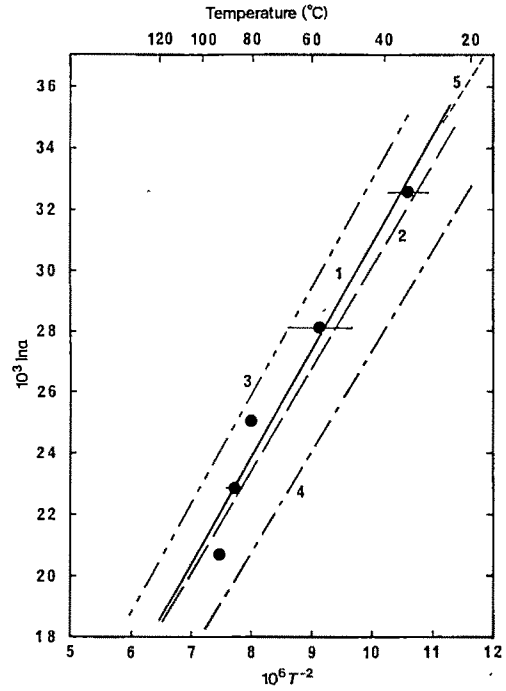


Fig. 1 Comparison of the oxygen isotope fractionation factor between amorphous silica and water with those previously reported for quartz. ●, Present results. Line 1, the least square fitting in the present study. Line 2, from refs 1, 2. Line 3, from Taylor (cited in ref. 3) Line 4, from ref 4 Line 5, from ref. 12

The dehydration of hydrous silica changes the oxygen isotope ratio of the silica remaining[6] In this study, however, air-dried amorphous silicas were preheated in vacuum at 200 °C for 3 h before the reaction with fluorine gas, for the following reasons.

The SiO_2 content calculated from the amount of oxygen extracted by fluorination ranged from 88 to 95 wt%. These reductions (5 to 12 wt%) in content correspond to the weight loss when the amorphous silicas were heated at 1,000 °C. Therefore, these reductions must be due to dehydration of silica during preheating. In that case, the water in the amorphous silica can be completely removed at 200 °C, and much of the water should be removed at temperatures below 200 °C. The water removed at such lower temperatures may be simply adsorbed water, or water weakly combined with silica, the dehydration causes very little change in the oxygen isotope ratio of silica remaining[12].

As shown in Table 1, the amorphous silicas precipitated on the different materials both at P-1 and at P-4 have the same $\delta^{18}O$ value at each point. This indicates that the difference in the character of surface of the materials does not influence the oxygen isotope fractionation between silica and water. Scanning electron microscopy confirms that the difference in the character of surface has no effect on the morphology of amorphous silica.

The fractionation factors (α) between amorphous silica and geothermal water are given by

$$\alpha = (1+10^{-3}\delta^{18}O_{silica})/(1+10^{-3}\delta^{18}O_{water})$$

as shown in Table 1. The relationship of $\ln\alpha$ to reciprocal temperature square is almost linear, as seen in Fig. 1, and is approximated by

$$10^3 \ln\alpha = 3.52(10^6 T^{-2}) - 4.35$$

This relationship is similar to that of the equilibrium fractionation factor between quartz and water. It is especially close to the extrapolated relationship between quartz and water as determined experimentally by Clayton *et al.*[1] and Matsuhisa *et al*[2]

Knauth and Epstein[6] estimated the fractionation factor between quartz and water to be 1.039 at 0 °C using the observed oxygen isotope ratio of a microcrystalline quartz in an oceanic sediment. This value is close to the relationship determined by Kawabe[4] and lower by about 5‰ than the present result. Labeyrie's[12] measurement of the fractionation between silica in sponges and diatoms and water is very close to the present one.

An interesting problem is whether the oxygen isotope fractionation takes place among polymorphs of silica. In the case of α and β quartz, for instance, fractionation of 0.8 to 1.5‰ is theoretically predicted[3,4], although no evidence has been found from experimental studies[2]. In the case of amorphous silica, the problem is whether a definite fractionation factor is available, because amorphous silica has no definite crystal structure. Nevertheless, the present result suggests that the extent of fractionation between amorphous silica and quartz may be smaller than the extent of variation in the equilibrium fractionation factors reported in the literature for the quartz-water system.

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1 Clayton, R N, O'Neil, J R & Mayeda, T K *J geophys Res* **77**, 3057-3067 (1972)
2 Matsuhisa, Y, Goldsmith, J R & Clayton, R N *Geochim cosmochim Acta* **43**, 1131-1140 (1979)
3 Shiro, Y & Sakai, H *Bull chem Soc Jap* **45**, 2355-2359 (1972).
4 Kawabe, I *Geochim cosmochim Acta* **42**, 613-621 (1978)
5 Bottinga, Y & Javoy, M *Earth planet Sci Lett* **20**, 250-265 (1973)
6 Knauth, L P & Epstein, S *Earth planet Sci Lett* **25**, 1-10 (1975)
7 Brown, K L & McDowell, G D *Proc 5th N Z Geothermal Workshop*, 157-161 (University of Auckland, Auckland, 1983)
8 Shimono, T, Isobe, T & Tarutani, T *J Chromatogr* **258**, 73-80 (1983)
9 Ichikuni, M, Tsurumi, M & Kadoya, H *Chikyukagaku* **17**, 137-141 (1983) (in Japanese)
10 Kita, I & Matsubaya, O *Rep Res Inst Underground Resour, Min College, Akita Univ* No 48, 25-34 (1983) (in Japanese)
11 O'Neil, J R & Epstein, S *J geophys Res* **71**, 4955-4961 (1966)
12 Labeyrie, L *Nature* **248**, 40-42 (1974)

Major Asian aeolian inputs indicated by the mineralogy of aerosols and sediments in the western North Pacific

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Recent atmospheric chemistry studies have shown that substantial quantities of soil material are being transported out of Asia and across large regions of the North Pacific[1,2]. In the central North Pacific, the estimated[1] ($6-12\times10^{12}$ g yr^{-1}) and measured[2] (20×10^{12} g yr^{-1}) deposition rate of mineral aerosols could account for a substantial fraction of the non-biogenic portion of deep-sea sediments in this region, where sedimentologists have previously estimated that 75–95%[3,4] of the surface sediment is derived from atmospheric dust fallout. The quantitative importance of wind-transported (aeolian) material to sedimentation in the North Pacific would be more firmly established if it could be shown that the composition of the present-day soil aerosol is similar to that of mineral particles in the underlying sediments, which have been deposited over a period of thousands of years. Here we compare the compositions of soil aerosols and sediments collected from the western North Pacific and find that their mineralogies are identical, except for one mineral. This supports the hypothesis that the sediments are heavily impacted by aeolian sources.

The mineralogy of the <2-μm size fraction of both aerosols and sediments was determined by X-ray powder diffractometry using methods developed specifically for <2-μm sediments[5]. This size fraction should yield a substantial fraction of the total aerosol mass, which has a median diameter of ~2 μm[6,7]. Aerosols of this size have a very small settling velocity in the atmosphere; hence, they will have a relatively long residence time and their composition will be little affected by changes due to differential settling of different-sized mineral constituents.

The sediment samples were taken from the tops of 12 piston cores (Table 1*b*) collected during R/V *Vema* cruise 36-12 (Fig. 1). Core sites are located on the seaward side of the western Pacific trenches in an area where there is no evidence of turbidites[8] or of inputs from ice rafting[9]. On the basis of their locations, mineralogy and stratigraphy, it has been suggested that the inorganic (non-biogenic)-mineral component of the sediments is predominantly aeolian in origin[10,11]. Although there are differences in sedimentation rates between the areas sampled by coring, there is no evidence of extensive reworking of sediments by bottom currents or erosion at the sites[12]. Finally, the average age of the sediments used for this study is <10 kyr[13]. Thus, they probably have not been influenced by the major climatic changes associated with Pleistocene glaciations, with the possible exceptions of the three cores 33P, 45P and 49P, which show ages of 12–17 kyr[13].

The mineral aerosols were collected with two 1-m^2 nylon mesh samplers[14] aboard the DV *Glomar Challenger* during the Deep-Sea Drilling Project (Table 1*a*) in the western North Pacific (Fig. 1). Of the samples collected in this region, the six largest were selected for analysis. Previously, there has been concern that the collection efficiency of the meshes may vary with particle size so that the samples would not be representative of the total aerosol[14–16]. In a recent study, however, the <2-μm mineralogies of soil aerosols collected concurrently with filters and with meshes were found to be identical except for smectite, which was underestimated in the mesh samples relative to the filter samples by a factor of about two (M.B. and J.M.P., in preparation).

Sediment samples were treated with sodium acetate (buffered to *p*H 4.8 with acetic acid) to remove $CaCO_3$, with 0.4 M Na_2CO_3 to remove biogenic silica (opal), and with a Na dithionite/Na citrate solution to remove amorphous iron and manganese oxides[17]. Subsequent treatment and analysis was identical for both aerosol and sediment samples. Bulk aerosol sample masses ranged from 0.4 to 4.3 g per sample. Part (200–500 mg) of each sample was saturated with 1.0 M $MgCl_2$ (ref. 5). Size separations were made by wet-sieving at 20 μm diameter. The <2-μm fraction was isolated by a settling procedure. To increase the precision of X-ray analysis, an internal standard of 10 wt% <2-μm talc was added to each <2-μm sample[5], which was then deposited as a slurry on a 0.45-μm silver filter and X-irradiated[5] (see Table 3). The peak areas for illite, smectite, kaolinite, chlorite, quartz and plagioclase were converted to wt% using the weighting factors determined by Heath and Pisias[5] for modern North Pacific sediments. Amphibole concentrations were undetectable or present in only trace amounts. The sum of the wt% of the major mineral components is equivalent to the percentage of diffracting phases in the sample. The average

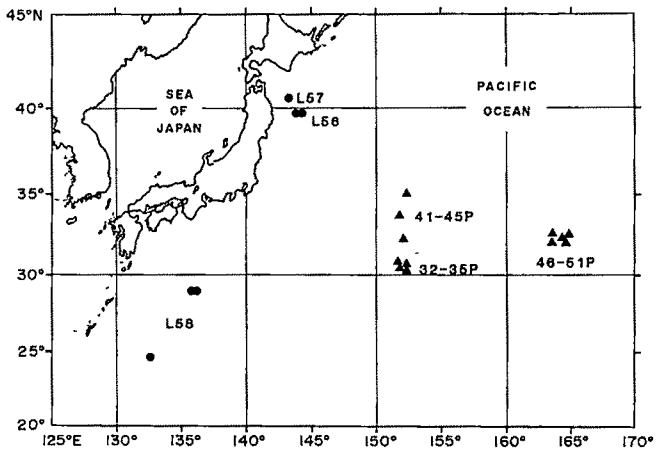


Fig. 1 Collection sites of *Glomar Challenger* aerosol samples and Vema 36/12 32P–51P sediment cores. The circles and triangles represent the aerosols and sediments, respectively. (See Table 1.)

Table 1 Aerosol and sediment samples

Aerosol mesh samples collected aboard the *Glomar Challenger*

Sample Leg	Mesh	On site	Date sampled	Sampling period (h)	Ship location
56	2	434B	23–24 September 1977	25.5	39°45′ N 144°06′ E
56	3	434B	27–28 September 1977	30.5	39°45′ N 144°06′ E
57	1	438A	22–24 October 1977	52.3	40°38′ N 145°33′ E
58	2	442	16–18 December 1977	47.0	28°59′ N 136°04′ E
58	3	442A	18–20 December 1977	50.2	28°59′ N 136°04′ E
58	17	446	20–22 January 1978	47.5	24°42′ N 132°47′ E

Piston core samples from *Vema* 36-12 cruise

Univ. Rhode Island accession no.	Core	Depth (cm)	Latitude	Longitude
58,752	V36/12-32P	6–8	30°55.3′ N	151°37.1′ E
58,753	V36/12-33P	10–12	30°20.7′ N	152°16.4′ E
58,754	V36/12-34P	10–12	30°45.6′ N	152°16.2′ E
58,755	V36/12-35P	8–10	30°30.1′ N	151°48.7′ E
58,756	V36/12-41P	10–12	32°21.1′ N	152°03.1′ E
58,757	V36/12-43P	10–12	33°45.0′ N	151°45.8′ E
58,758	V36/12-45P	12–14	35°05.6′ N	152°16.4′ E
58,759	V36/12-46P	3–5	32°41.1′N	163°31.7′ E
58,760	V36/12-47P	2–4	32°06.0′ N	164°32.0′ E
58,761	V36/12-48P	3–5	32°08.9′ N	163°28.6′ E
58,762	V36/12-49P	8–10	32°24.0′ N	164°16.0′ E
58,763	V36/12-51P	2–4	32°38.0′ N	164°46.0′ E

Average water depth, 5,100 m (ref. 8).

Table 2 Analytical variability caused by machine fluctuations and laboratory methods

	Qtz	Plag.	Smec.	Illite	Kaol.	Chl.	I/Q	K/C	I/K
$\bar{x}$	8.8	10.1	0.8	42 0	15.5	3.2	4.81	4.84	2.77
s.d.	0.52	1.68	0.64	2.35	2.15	0.27	0 47	0 87	0.51
r	8.2–9.4	7.5–12.2	0.2–2.0	37.7–45.6	12.3–18.8	2.9–3.6	4.14–5.56	3.61–6.06	2.01–3.51

Aerosol sample L58K3 (<2-μm size fraction) was analysed eight times Data are normalized to 80.4% diffracting Qtz, quartz; Plag , plagioclase, Smec., smectite; Kaol , kaolinite; Chl., chlorite; I/Q, illite/quartz ratio; K/C, kaolinite/chlorite ratio, I/K, illite/kaolinite ratio; $\bar{x}$, mean, r, range.

Table 3 Comparison of mean composition of <2-μm sample size fraction

	Qtz	Plag.	Smec.	Illite	Kaol	Chl.	I/Q	K/C	I/K
Aerosols ($n=6$)									
$\bar{x}$	10.5	11.2	1 1	39.5	15.5	2.7	3.81	5.81	2.66
$s_{\bar{x}}$	0.49	0 86	0 26	1 92	1.16	0.15	0.29	0.38	0.29
Sediments ($n=12$)									
$\bar{x}$	9.9	9.7	3.1	38.7	16.4	3.0	3 90	5.72	2 61
$s_{\bar{x}}$	0 27	0.41	0 44	0.89	1.18	0.15	0 15	0 49	0.24

Samples were X-irradiated on a Phillips Norelco X-ray diffractometer using Ni-filtered CuKα radiation at 40 kV and 25–40 mA. Samples were scanned over the range 3–30° (2θ) at a rate of 1 deg min^{-1}. Weight percentages are normalized to maximum percentage diffracting, 80.4%

percentage of diffracting phase in the aerosols was 55.7% and that for the sediments was somewhat higher, 71%, because more amorphous component was removed before X irradiation. The residual fraction (100 − % diffracting) is composed of poorly crystalline minerals and X-ray amorphous phases in the sample[5]. To facilitate the comparison of the different samples, the mineral percentages were normalized to the maximum diffracting percentage, 80.4%.

The variability in X-ray clay mineral analysis can be very high because the analyses are extremely sensitive to sample preparation and instrumental effects. Multiple runs were performed of many samples to assess the analytical precision and, in one case, an aerosol sample was subsampled and analysed eight different times to determine the error due to sample preparation and day to day variability in the X-ray diffractometer. The precision is good with the exception of smectite (Table 2).

The mineralogies of the mesh samples and the sediments are strikingly similar (Table 3). The differences in composition between the two materials are less than the analytical variability except for smectite. Statistical f-tests and Student's t-tests indicate that the aerosols and sediments are statistically identical (99% confidence level) except for smectite. This difference for smectite is probably not significant, because of the relatively low precision in smectite analysis, the small percentages involved and the possible undersampling of smectite by the mesh samplers. The amount of biogenic opal and other non-terrigenous components in the bulk sediment ranges from 2–20% in the area of our cores[10], which, with the above evidence, suggests that the remaining 80–98% of the sediment is aeolian in origin and that aeolian inputs dominate the deep-sea sedimentation there. The location of our samples, atmospheric haze data[18] and the results of North Pacific dust trajectory studies (J. T. Merrill *et al.*, in preparation) suggest that the arid regions of Asia are the source of the mineral aerosol.

The rate of mineral aerosol deposition in this region seems quite high. The total dust deposition rates measured recently at island stations 3,500–5,600 km east of the Vema core locations are 0·064 g cm^{-2} kyr^{-1} at Midway and 0.043 g cm^{-2} kyr^{-1} at Oahu[1]. Average sedimentation rates for the cores vary from 0.86 cm kyr^{-1} for the cores near 152° E to 0.67 cm kyr^{-1} for those near 164° E[13] and dry sediment bulk densities average 0.3–0.4 g cm^{-3} for both areas[19]. If ~80% of this material is aeolian, the average mass accumulation rates of aeolian material for the two core areas are 0.24 g cm^{-2} kyr^{-1} and 0.18 g cm^{-2} kyr^{-1}. These higher rates may be attributable to the fact that the Vema sites are closer to the Asian sources where dust fluxes could be substantially higher. We have estimated a mean dust concentration for the core locations using that measured in the Sea/Air Exchange (SEAREX) programme's North Pacific island network[2,6] and Asian dust concentrations measured in a similar network in Japan (M. Uematsu, personal communication). A semilogarithmic interpolation between these networks yields a mean mineral aerosol concentration of 6–10 μg m^{-3}. On the basis of deposition models developed in the SEAREX studies[1], we estimate the mineral aerosol deposition rate at the core sites to be 0.2–0.3 g cm^{-2} kyr^{-1}, in good agreement with the measured aeolian sediment accumulation rates. These results suggest that the present rate of dust transport out of Asia is representative of transport over the past few thousand years. It follows that the non-biogenic mineral components in North Pacific sediments could be a good indicator of palaeoclimatic conditions such as aridity, wind velocity and large-scale atmospheric circulation patterns.

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1 Uematsu, M *et al J geophys Res* **88**, 5343–5352 (1983)
2 Uematsu, M , Duce, R A & Prospero, J M *J atmos Chem* (submitted)
3 Windom, H L *Bull geol Soc Am* **80**, 761 (1969)
4 Leinen, M & Heath, G R *Palaeogeogr Palaeoclim Palaeoecol* **36**, 1–21 (1981)
5 Heath, G R & Pisias, N G *Clays Clay Miner* **27**, 175–184 (1979)
6 Duce, R A *et al Science* **209**, 1522–1524 (1980)
7 Kadowaki, S *Envir Sci Technol* **13**, 1130 (1979)
8 Heath, G R , Pisias, N G & Lopez, C in *Subseabed Disposal Program a Rep* Vol 2 (ed Hinga, K) 389–468 (SAND82-0664/II, US Government Printing Office, 1982)
9 Ninkovitch, D & Robertson, J H *Earth planet Sci Lett* **27**, 127–136 (1975)
10 Pisias, N G & Leinen, M in *Milankovitch and Climate* (eds Berger, A *et al*) 307–330 (Reidel, Dordrecht, 1984)
11 Leinen, M & King Walsh, T in *Subseabed Disposal Program a Rep* Vol 2 (ed Hinga, K) (SAND83-0664/II, in the press)
12 Damuth, J E , Jacobi, R D & Hayes D E *Bull geol Soc Am* **94**, 381–395 (1983)
13 Barton, C & Sopher, E in *Subseabed Disposal Program a Rep* Vol 2 (ed Hinga, K) 235–334 (SAND82-0664/II, 1981)
14 Prospero, J M & Bonatti, E *J geophys Res* **74**, 3362–3371 (1969)
15 Prospero, J M & Nees, R T *Science* **196**, 1196–1198 (1977)
16 Delany, A C *et al Geochim cosmochim Acta* **31**, 885–909 (1967)
17 Jackson, M L *Soil Chemical Analysis—Advanced Course* (Jackson, Madison 6, Wisconsin, 1956)
18 Prospero, J M in *The Sea* Vol 7 (ed Emiliani, C) 801–874 (Wiley, New York, 1981)
19 Dadey, K thesis, Univ Rhode Island (1984)

Thermogenic hydrocarbons in surface sediments of the Bransfield Strait, Antarctic Peninsula

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Antarctica is the only continent from which petroleum deposits have not been encountered, despite the occurrence of potential hydrocarbon basins onshore and around its margins. We have discovered thermogenic hydrocarbons in unconsolidated Recent sediments from the King George Basin, Bransfield Strait, west Antarctica. These sediments possessed a marked petroliferous smell throughout the length of the 8.6-m gravity core. The basin sediments are glacial marine deposits dominated by turbidites and contain abundant autochthonous organic matter as a result of high seasonal primary productivity in this most fertile part of the circumpolar ocean[1]. The maturation of this material into hydrocarbons may have been accelerated by the high geothermal gradient in the basin, associated with back-arc spreading created by the subduction of the Drake Plate into the South Shetland trench[2]. Sediments in this rifted basin are frequently intruded by volcanic sills and dykes[3] giving rise to acoustic features shown in Fig. 1. The presence of these thermogenic hydrocarbons provides the first demonstration of active source rocks in Antarctica.

On core retrieval, the liners were immediately cut into 1-m sections and ~500 g of wet sediment removed to a freezer and kept at −80 °C until analysed (within 24 h). The analytical procedure for the molecular and stable isotope composition of the hydrocarbon gases is similar to that given elsewhere[4]. The bitumen fraction (C_{15+}) was extracted with CH_2Cl_2 by soxhlet, partitioned by liquid chromatography (LC) and analysed by gas chromatography/mass spectrometry[5]. Interstitial waters were pressure filtered immediately after coring. Total dissolved carbonate (ΣCO_2) was determined by gas chromatography following acidification and stripping of the sample and dissolved sulphate (SO_4^{2-}) was determined gravimetrically.

Methane yields are low in the uppermost 3 m (< 32 parts per 10^9 wt gas/wt sediment) then increase to a maximum of 153 parts per 10^9 at 450 cm (Fig. 2). The distribution of ethane to pentane hydrocarbons (C_{2+}) roughly covaries with methane, with a maximum of 197 parts per 10^9 in the sediments (Table 1*a*). In addition, some of the gas chromatograms from the deeper samples reveal C_6 and C_7 hydrocarbons in concentrations even greater than those of C_1–C_5. Unsaturated hydrocarbons, ethene and propene were present in only minor amounts in comparison to the *n*-alkanes. Within the C_{2+} fraction, the concentration tends to increase corresponding to carbon number below 650 cm (Table 1*b*). Relative to methane the C_{2+} gas fraction is greater at depth, with % wetness ratios ($[\Sigma C_{2-5}/\Sigma C_{1-5}] \times 100$) reaching values of 39%. The high % wetness here is related to hydrocarbon formation via thermal processes, rather than via diagenetic processes which may produce minor quantities of C_{2+} (ref. 6).

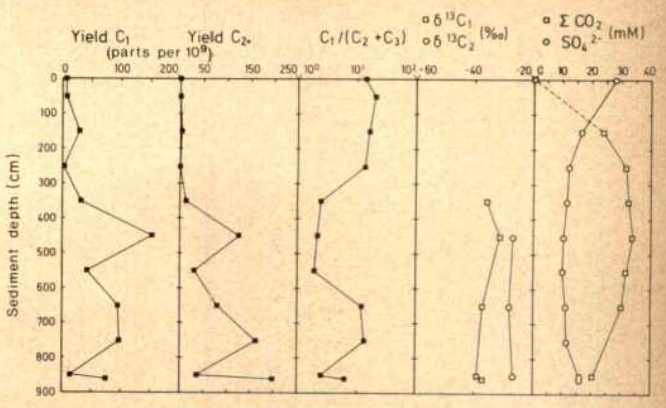


Fig. 2 Hydrocarbon gas concentrations of methane (C_1) and ethane through pentane (C_{2+}) increases with sediment depth in core 1140. Thermogenic gas signature confirmed by $C_1/(C_2+C_3)$ and by $\delta^{13}C_1$ and $\delta^{13}C_2$ values in the thermal range. The presence of sulphate throughout the core precludes *in situ* biogenic methane production. Remineralization of organic matter appears to cease below 300 cm, possibly owing to lower TOC contents (<0.9%).

Methane produced microbially is frequently observed in strongly reducing sediments, with associated minor C_{2+} concentrations 2–4 orders of magnitude lower. Several other locations in the Bransfield Strait exhibit bacterial methanogenesis in the sediments, but only under strongly reducing conditions where the interstitial dissolved sulphate has been exhausted. Dissolved sulphate in core 1140-1 showed evidence of reduction, but SO_4^{2-} persisted in large amounts throughout the core (Fig. 2), precluding bacterial methanogenesis as a source of the methane. In core 1140-1, methane contents are lower and C_{2+} contents much higher than those found in other sediments of the King George Basin exhibiting bacterial methanogenesis.

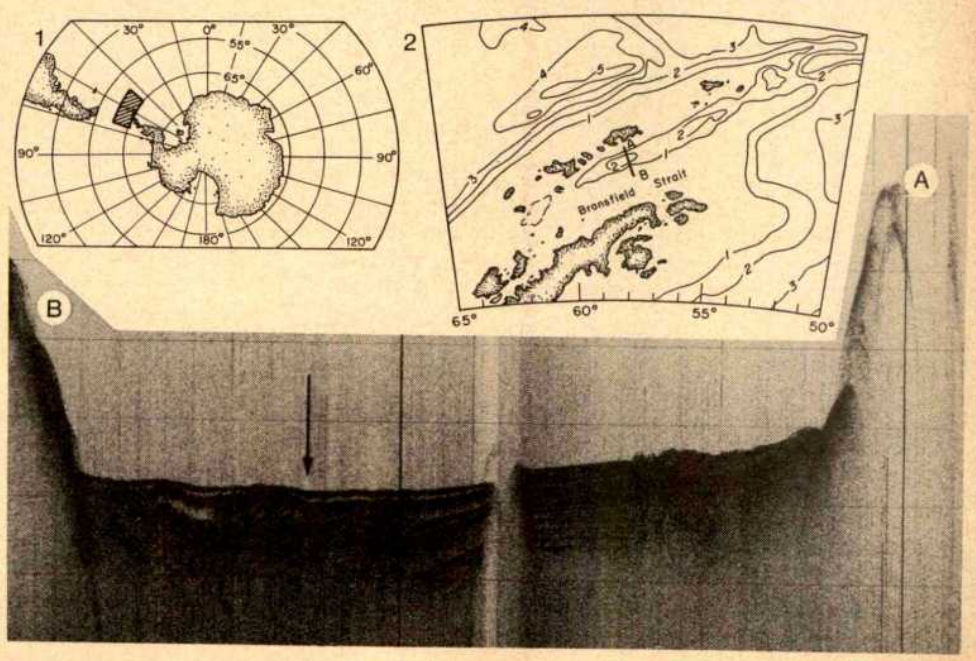


Fig. 1 Section of 3.5-kHz sediment profile showing northern (A) and southern (B) limit of King George Basin. Inset 1 is a polar projection of Antarctica showing the investigation area; inset 2 gives the bathymetry (1,000-m isobaths) and 3.5-kHz track between King George Island and the Antarctic Peninsula. Core 1140 (indicated by arrow) was taken in fine-grained turbid sequences of hemipelagic muds overlying an acoustic feature where loss of signal occurs below 5-m sediment depth. The steep basalt intrusion north of the station can be seen (centre of photo).

Table 1 Hydrocarbon gas and interstitial fluid analyses from core 1140-1, King George Basin

a

Depth (cm)	Yield C_1 (parts per 10^9)	Yield C_{2+} (parts per 10^9)	$C_1/(C_2+C_3)$	$\delta^{13}C_1$ (‰)	$\delta^{13}C_2$ (‰)	$[SO_4^{2-}]$ (mM)	ΣCO_2 (mM)
0	6	0	25.9	ND	ND	ND	ND
50	8	0	38.4	ND	ND	ND	ND
150	29	2	33.8	ND	ND	16.13	23.5
250	3	0	25.5	ND	ND	11.86	31.3
350	32	13	6.1	−35.8	ND	11.24	32.2
450	153	125	4.7	−31.4	−26.8	10.11	33.5
550	43	31	4.1	ND	ND	9.77	31.4
650	96	80	27.4	−37.1	−28.0	10.95	29.7
750	99	162	28.9	ND	ND	11.31	ND
850	15	36	5.2	−39.0	−26.8	15.68	20.1
860	76	197	14.5	−37.1	ND	15.68	ND

b

Depth (cm)	% Hydrocarbons						
	C_1	C_2	C_3	iso-C_4	n-C_4	iso-C_5	n-C_5
0	94.1	3.6	0.0	0.0	0.0	0.0	0.0
50	97.4	2.5	0.0	0.0	0.0	0.0	0.0
150	96.8	2.1	0.7	0.0	0.0	0.0	0.0
250	96.2	3.7	0.0	0.0	0.0	0.0	0.0
350	85.9	4.9	9.1	0.0	0.0	0.0	0.0
450	76.1	12.1	4.2	1.9	1.5	2.6	0.7
550	76.5	13.2	5.3	1.7	1.1	0.8	0.2
650	81.7	1.8	1.1	2.7	2.8	6.6	2.5
750	71.6	1.8	0.6	0.9	1.4	15.4	7.5
850	60.6	9.5	2.0	2.9	2.3	11.2	11.2
860	61.3	2.4	1.7	3.4	2.7	15.5	12.6

ND, not determined.

Carbon isotope ratios of methane and ethane were measured on the samples with higher yields and are reported in the usual δ notation relative to the PDB standard. Methane in the deeper core sections is slightly more depleted in ^{13}C (−37.1 to −39.0‰, Fig. 2) than in those nearer the surface (−31 to −35‰). Carbon isotopes of ethane were more uniformly distributed between −26.8 and −28.0‰. Thermal maturities of natural gases can be estimated using the relationship of carbon isotope ratios of methane and ethane to vitrinite reflectance[7,8]. Based on such relationships (M.J.W. and E. Faber, in preparation), the inferred maturity of the gases in the deeper core section ranges between 1.4 and 1.8% R_o (equivalent) for methane and between 1.5 and 1.7% R_o (equivalent) for ethane (Fig. 3). The good agreement between them supports the idea that the methane and C_{2+} hydrocarbons in these sediments have a similar source and thermal history. The average maturity estimate of 1.6% R_o (equivalent) of the gases places them in a late thermal stage, possibly in the condensate range.

A combination of the molecular and carbon isotope composition in the classification diagram of Bernard *et al.*[9] (Fig. 4) shows the gases to be of the late thermal associated type. The possibility of an admixture of an overcooked dry methane with an associated wet gas can be discounted because of the agreement in maturity between the C_1 and C_2 isotope data. There is no evidence of secondary alteration effects such as anaerobic oxidation in the deeper sediments. Although the low methane concentrations in the uppermost sediments may have been influenced by oxidation, the expected associated relative enrichment in C_{2+} was not observed.

The composition of solvent extracts further indicates the presence of thermal hydrocarbons in these surface sediments. The five extracted sediment samples yielded 82–320 p.p.m. (dry wt) soluble material (Table 2), which is relatively high for Recent sediments with 0.62% and 0.87% total organic carbon (TOC). The total extract/total organic carbon ratio (mg extract/g C_{org}) increases from 13 at the surface to 46 at the bottom. The lower three samples had sufficient extract for LC partitioning of which more than 50%, hetero-components and other polar compounds, was irreversibly bound to the LC column. Nevertheless, hydrocarbon concentrations are found up to 141 p.p.m. at 860 cm, which is somewhat anomalous for this diagenetic setting.

The mixture of hydrocarbons from a deeper more mature source with autochthonous bitumen is expressed most clearly in the samples from 350 and 650 cm. The saturated C_{15+} n-alkanes are dominant between C-numbers 25 and 31, apart from the deepest sample (860 cm) where phytane constitutes the major component. Where detectable, phytane is more concentrated than pristane signifying a reducing depositional environment[10] consistent with the interstitial water chemistry. n-Alkanes show an odd–even predominance (OEP) of 1.32–1.50 (Table 2), excepting the sample at 350 cm where the OEP is 1.04.

There is a relatively high contribution of rearranged steranes at 650 cm, which would not be expected in immature Recent sediments. Furthermore, the sediment from 350 cm possesses a hopane signature characteristic of many oils. In particular, the ratio of the 17-α(H)-hopane, C_{31} 22S/22R, is >1 at 350 cm (Table 2). With these exceptions, the extracts are typical of

Table 2 Analysis of C_{15} hydrocarbons from core 1140-1

Depth (cm)	TOC (%)	Extract (p.p.m.)	Saturated (p.p.m.)	Aromatic (p.p.m.)	Heterocyclic (p.p.m.)	OEP	tr/st	Ts/Tm	C_{29}-20S/ C_{29}-20R	C_{31}-22S/ C_{31}-22R
5	0.62	82	ND	ND	ND	1.32	5	0.53	0.43	0.64
150	0.85	150	ND	ND	ND	1.57	7	0.36	0.21	0.67
350	0.87	257	84	40	108	1.04	5	0.81	0.67	1.17
650	0.85	189	17	35	114	1.51	4	0.54	0.43	0.47
860	0.70	320	44	98	113	1.41	1	0.11	ND	0.43

OEP, odd–even predominance (n-alkanes); tr/st, triterpane/sterane ratio; Ts/Tm, 18α(H)-trisnorhopane/17α(H)-trisnorhopane. ND, not determined.

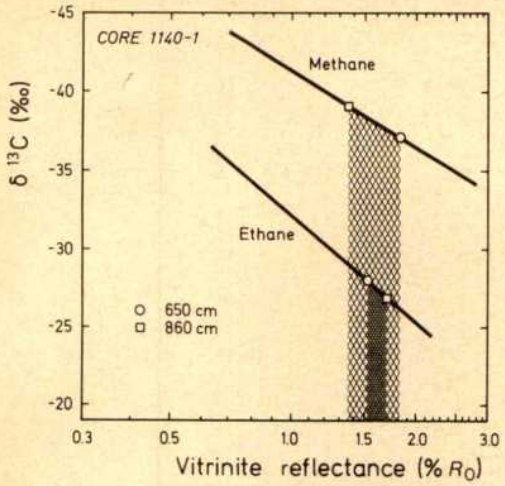


Fig. 3 Maturity of source organic matter generating the hydrocarbon gases is estimated using the empirical relationship between $\delta^{13}C$ values of C_1 and C_2 and maturation levels (M.J.W. and E. Faber, in preparation). These gases are formed in the range of the late-mature stage corresponding to a mean vitrinite reflectance of ~1.6% R_o equivalent.

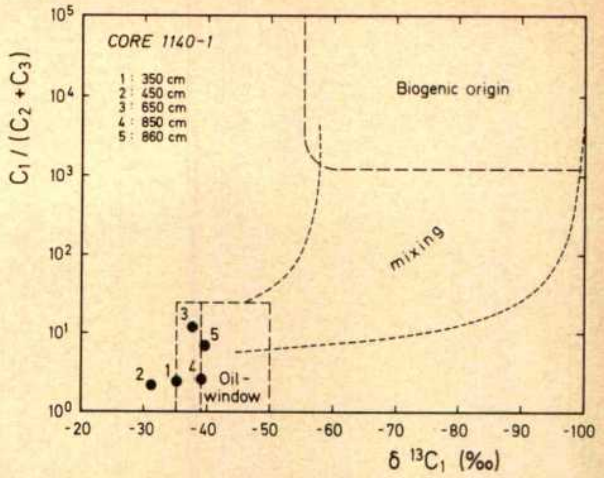


Fig. 4 Natural gas genetic classification diagram after Faber *et al.*[25] clearly showing late-stage thermal character of the hydrocarbon gases measured. There is no evidence for biogenic or mixed gases in these cored sediments.

immature organic material, with contributions of terrestrial organic material, shown by the OEP, of the higher carbon number (C_{24+}) *n*-alkane, and low contents of rearranged steranes (5-β sterane is less abundant than 5-α steranes and 20S-ethylcholestane is less than 20R-ethylcholestane). In addition, the Ts/Tm triterpane ratios (18-α(H)/17-α(H)-trisnorhopane[11]) of these samples are <1, the $C_{29}+C_{30}$ moretane contents are relatively high and C_{31}-22S of 17-α(H)-hopanes is less than C_{31}-22R (Table 2). Thus, although the degree of impregnation is somewhat low in samples at 350 and 650 cm, there are positive indications that heavier thermogenic hydrocarbons have migrated into these sediments.

The maturity of the migrated hydrocarbon component in these samples is roughly estimated by the OEP and biomarkers to be early mature ($<0.5\%$ R_o equivalent). The discrepancy between the maturity of the light hydrocarbon gases as determined by carbon isotopes (1.6% R_o equivalent) and that of the allochthonous C_{15+} hydrocarbons based on biomarkers ($<0.5\%$ R_o equivalent) and the *in situ* vitrinite reflectance ($<0.2\%$ R_o) would suggest that the gases come from more mature organic sources possibly more deeply buried than those generating the heavier bitumens. Such gases could assist in the transport of the bitumens acting for example as a solvent (compare ref. 12) The limited number of cores makes it difficult to locate the source of the hydrocarbons, or to define their regional surface expression. Coring attempts at a second station nearby (1185-1) were frustrated by basalt, but analyses of a small sample of sediment and basaltic debris remaining in the core catcher showed clear traces of C_{2+} hydrocarbon gases.

The thermogenic hydrocarbons were cored in acoustically-'turbid' horizons in the sediments of the eastern edge of the basin (Fig. 1). The cause of the acoustic turbidity is unknown. The measured light gas concentrations are not even close to the solubility limit necessary to form free gas bubbles[13] and the heavier hydrocarbons (C_{5+}) are liquid at the *in situ* pressure and temperature. In addition, the sediments cored are within the stability zone of gas hydrates[14]. If the gas contents were high enough to permit a free gas phase, this gas would be incorporated into a hydrate structure, though it is possible that gases at depths greater than those of the cored sediment may be trapped beneath, or in, hydrate caps[15]. Such preferential removal, or trapping, of the light (C_1-C_4) gases could be one explanation for the relatively high C_{5+} contents in the surface sediments. There were, however, no indications direct or indirect, of hydrates in this acoustically-turbid zone in the 3.5-kHz trace (Fig. 1). Such seismic indicators of gas hydrates are not always visible and because of the shallow coring depth (860 cm, maximum) the possibility of hydrates cannot be disregarded.

Another explanation for the absence of reflectors would be the presence of oligostromes or mud diapirs, which frequently occur in areas of rapid subsidence with undercompaction and greater heat flow. Upward-transported sediments or fluids could carry the hydrocarbon gases and even bitumens acquired at greater depth[16]; vertical fluid expulsion or flushing (mentioned above) could take place in the area of coring resulting in the decrease in bitumen and higher relative molecular mass bias ($>C_{24+}$) close to the surface, despite no change in organic facies or maturity; the lower methane concentrations in the uppermost sediments could likewise be affected. Unfortunately, surface morphology and sedimentology do not substantiate this explanation.

The King George Basin is a tectonically active zone which has extensional features such as normal dip-slip faults and basalt intrusions located close to station 1140-1 (Fig. 1, centre); higher geothermal gradients would be expected in such a geological setting. The Guaymas Basin is a comparable tectonic setting with a high geothermal gradient where hydrocarbons have been thermally generated from mature organic matter in younger than normal sediments in the proximity of intrusions[17].

Previous offshore drilling along Antarctic continental margins has been limited to Leg 28 of the Deep Sea Drilling Project in the Ross Sea and off the Shackleton Ice Sheet[18]. At three sites in the Ross Sea, minor amounts of ethane and higher homologues were measured along with substantial quantities of methane[19]. Carbon isotope data reported for the methane (−68 to −79‰) and the strong sulphate reduction at these sites[18,20] would indicate microbial methanogenesis. The contribution from thermogenic sources in this region, based solely on the molecular composition data, is uncertain. Recently, investigations on the opposite side of Antarctica, off Wilkesland, have reported bacterial gas in low concentrations, but no thermogenic gas was indicated[21].

Hydrocarbon seeps are common for most of the world's major petroleum accumulations many of which are also trapped in graben structures as in the Bransfield Strait[22]. The large-scale rift faults and igneous intrusions can create migration pathways to the surface for gaseous and liquid hydrocarbons. Several analogous cases have been reported, as for example, in California, Mexico, Sumatra and numerous locations extending along the Andes from Columbia to Terra del Fuego[23,24]. The hydrocarbon seepage in core 1140-1, however, is not on the same scale as those seeps and thus should be regarded as a micro seepage. Nevertheless, the data provide unambiguous geochemical evidence of active petroleum source rocks along the Antarctic continent.

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1. Barker, P. & Dalziel, I. W. D. *Geodynamics Ser.* **9**, 137–170 (1983).
2. Wefer, G. *et al. Nature* **299**, 145–147 (1982).
3. Zarikhin, I. P. *Antarctika* **21**, 63–69 (1982).
4. Faber, E. & Stahl, W. *Petroleum Geochemistry and Exploration of Europe* (ed. Brooks, J. M.) 51–63 (Blackwell, Oxford, 1983).
5. Wehner, H. & Teschner, M. *J. Chromatogr.* **204**, 481–490 (1981).
6. Whelan, J. K. & Hunt, J. M. *Init. Rep. DSDP* **63**, 775–784 (1984).
7. Stahl, W. J. & Carey, B. E. *Chem. Geol.* **16**, 257–267 (1975).
8. James, A. T. *Bull. Am. Ass. Petrol. Geol.* **67**, 1176–1191 (1983).
9. Bernard, B. B., Brooks, J. M. & Sackett, W. M. *Earth planet. Sci. Lett.* **31**, 48–54 (1976).
10. Powell, T. G. & McKirdy, D. M. *Nature phys. Sci.* **243**, 37–39 (1973).
11. Seifert, W. & Moldowan, J. M. *Geochim. cosmochim. Acta* **42**, 79–95 (1978).
12. Kvenvolden, K. A. & Claypool, G. E. *Bull. Am. Ass. Petrol. Geol.* **64**, 1078–1086 (1980).
13. Whiticar, M. J. *Dynamic Environment of the Ocean Floor* (eds Manheim, F. T. & Fanning, K. A.) 219–235 (Lexington Books, Massachusetts, 1982).
14. Miller, S. L. *Natural Gases in Marine Sediments* (ed. Kaplan, I. R.) 151–177 (Plenum, New York, 1974).
15. Kvenvolden, K. A. & McMenamin, M. A. *US Geol. Surv. Circ.* **825**, 1–11 (1980).
16. Dickey, P. A. & Hunt, J. M. *Am. Ass. Petrol. Geol. Mem.* **16**, 136–137 (1972).
17. Simoneit, B. R. T. & Galimov, E. M. *Chem. Geol.* **43**, 151–166 (1984).
18. Claypool, G. E. & Kvenvolden, K. A. *A. Rev. Earth planet. Sci.* **11**, 299–327 (1983).
19. McIver, R. D. *Init. Rep. DSDP* **28**, 815–817 (1975).
20. Mann, R. & Gieskes, J. M. *Init. Rep. DSDP* **28**, 805–814 (1975).
21. *Am. Ass. Petrol. Geol. Explorer* (May, 1984).
22. Harding, T. P. *Bull. Am. Ass. Petrol. Geol.* **68**, 333–362 (1984).
23. Link, W. K. *Bull. Am. Ass. Petrol. Geol.* **36**, 1505–1540 (1952).
24. Hunt, J. M. *Petroleum Geochemistry and Geology*, 1–617 (Freeman, San Francisco, 1979).
25. Faber, E., Rehder, S. & Stahl, W. *Erdöl Kohle Erdgas Petrochem.* **36**, 357–361 (1983).

Somatostatin immunoreactivity in neuritic plaques of Alzheimer's patients

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Senile dementia of the Alzheimer's type can be diagnosed with certainty only by examining neurofibrillary tangles and neuritic plaques under the microscope[1–4]. Recently, it has been suggested that the condition is linked to specific neurotransmitter systems[5], with a decline of cortical acetylcholine, choline acetyltransferase[5–8], cholinergic neurones projecting to the cortex[9–11], cortical noradrenaline content[12], locus coeruleus neurones[13,14] and cortical somatostatic content[15]. Using immunocytochemical methods[16], we here report that somatostatin-immunoreactive processes are present in neuritic plaques in human Alzheimer's specimens[17]. These data, as well as other reports of non-cholinergic changes[18,19], strongly imply that Alzheimer's disease cannot be linked exclusively to cortical cholinergic elements, as proposed previously[8]. Rather, our data on plaque and somatostatin co-localization and distribution patterns suggest that Alzheimer's neuropathology may involve primarily the loss of selective cortical neurones that are targets of the implicated transmitter systems and that plaque formation may result from the degeneration of presynaptic and postsynaptic neurites of large projection neurones in layers III and V. Given the neurochemically heterogeneous input to these cells, it is not surprising that several neutrotransmitter systems, one of which is somatostatin, are implicated in the pathology of Alzheimer's disease.

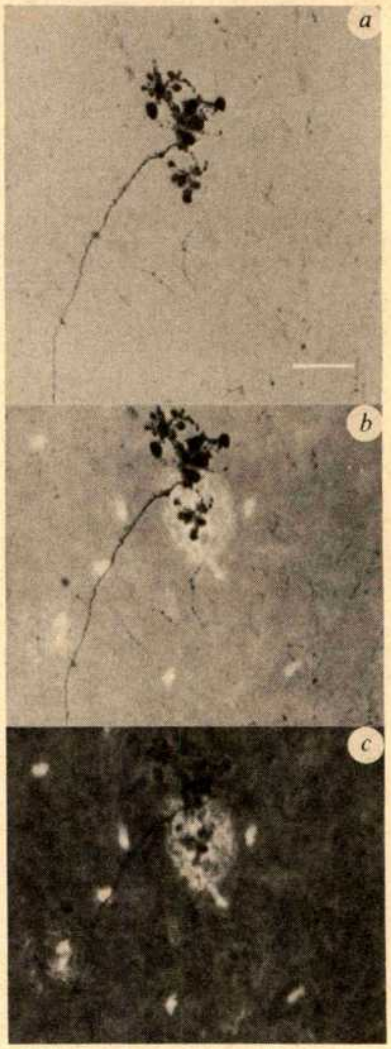


Fig. 1 Somatostatin immunoreactivity and a thioflavin-S-labelled neuritic plaque in an Alzheimer's patient: *a*, *b* and *c* describe the same microscopic field, representing the combined visualization of fluorescently labelled neuritic plaques (thioflavin-S) and somatostatin-immunoreactive profiles seen in conventional bright-field optics. *a*, Photomicrograph of this particular field in full bright field; *b*, the image obtained when viewed with fluorescence optics combined with low-intensity bright field; *c*, full fluorescence image alone. The somatostatin-immunoreactive profile (black) seems to be a cluster of degenerating boutons, many of which are bulbous and distended. In *b* and *c*, much of this pathological profile is associated with a fluorescent, amyloid-containing plaque with no clear core and thus possibly relatively immature[23,24]. Also, note that some of the bulbous profiles are outside the amyloid portion of the plaque. This complex may represent an early transitional stage where the plaque has not yet reached its full extent. Scale bar, 40 μm.

The present experiments examined normal adult, aged adult and Alzheimer cortices using antibodies against somatostatin-28 and somatostatin-28(1–12) (ref. 20). Specific cortical regions from postmortem samples of five clinically and neuropathologically confirmed Alzheimer brains and four brains from elderly patients with no neurological deficits were analysed. In some cases, sections were immunohistochemically stained to localize the prosomatostatin-derived peptides and then counterstained with thioflavin-S, a fluorescent stain that visualizes neuritic plaques and neurofibrillary tangles[21]. All Alzheimer brains contained neuritic plaques and neurofibrillary tangles in all regions stained.

In the human cortex, somatostatin antisera specific for either somatostatin-28 or somatostatin-28(1–12) show regional and laminar patterns similar to those observed in rodent cortex[16], but with a greater density of innervation. Somatostatin-positive

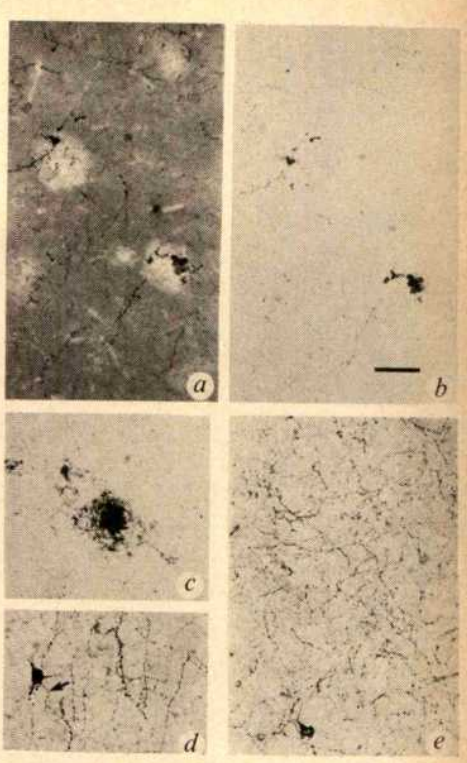


Fig. 2 Somatostatin immunoreactivity in human cortex. *a*, *b*, The same microscopic field in layer III of a section through the pre-central gyrus of a postmortem sample from an Alzheimer's patient. This section and *c* were stained immunohistochemically with S320 and counterstained with thioflavin-S. *a*, A fluorescence photomicrograph with the section exposed to fluorescence optics and low-intensity bright-field optics simultaneously. In this photograph, the fluorescent thioflavin-S-positive plaques show up as white puffs, somatostatin-immunoreactive fibres as darkly stained profiles. *b*, Exposed only to bright-field optics, thus the fluorescent plaques are not visible, but the immunoreactive diaminobenzidine-containing profiles are. Note that both of the large white plaques are associated with bulbous somatostatin-immunoreactive profiles and in both cases a labelled fibre can be seen entering the periphery of the plaque. *c*, Photomicrograph of a section stained with S320 through the superior frontal gyrus of a different Alzheimer's patient from that shown in *a* and *b*. Note the large coalescence of tangled somatostatin-immunoreactive fibres which have the appearance of a neuritic plaque; when viewed with fluorescence optics, this profile is not fluorescent and thus presumably does not contain amyloid. *d*, *e*, Photomicrographs of sections stained with 320, from postmortem samples of the frontal lobe of a neurologically normal 82-yr-old female. In *d*, the arrow points to a putative axon emerging from a labelled cell in layer III tentatively identified as a basket cell[16,26]. *e*, Normal density of somatostatin-immunoreactive fibres in layers II and III of frontal lobe from the same patient. Note the presence of highly arborized, varicose fibres. The cortical regions studied were the superior frontal gyrus, parahippocampal and inferior temporal gyrus, superior temporal gyrus, anterior cingulate gyrus, precentral gyrus, post-central gyrus and striate cortex.

Methods. Human tissue was obtained 4–16 h postmortem, immersed in fresh 4% paraformaldehyde phosphate buffer (*p*H 7.4) for 5 days, followed by graded sucrose solutions (12–18%) for several days and then sectioned by cryostat (40-μm sections). The sections, pretreated in 1% hydrogen peroxide for 15 min to remove intrinsic peroxidase activity[14], were stained immunohistochemically as reported previously[16], with antisera 309 and 320, immunochemically characterized previously[20]. S309 is directed against the N-terminal 12 amino acids of somatostatin-28 and thus recognizes both somatostatin-28 and somatostatin-28(1–12). The antigenic determinant of S320 is the C-terminus of somatostatin-28(1–12). The Glu-OH at the carboxy-terminus of the molecule is crucial for antigenicity so that neither somatostatin-28 or somatostatin-14 is read. Any N-terminal extension of somatostatin-28(1–12) will be recognized by S320 (see refs 16, 20). The tissues were then stained with thioflavin-S which has been shown to stain amyloid-containing profiles[21]. To stain with thioflavin-S, mounted tissue sections were hydrated in distilled H_2O for 10 min, followed by immersion in a filtered aqueous 1% thioflavin-S solution for 10 min. Sections were then differentiated and dehydrated through a series of alcohols (70% absolute) into xylene and coverslipped in Permount (Fisher). The tissues were viewed with a Zeiss microscope equipped for both fluorescence and bright-field optics. Scale bar in *b*, 60 μm (applying to all photomicrographs).

cell bodies and terminals exhibit a bilaminar distribution; for example, there are few labelled cells or fibres in layer IV, but much higher densities of labelled cells in layers II, superficial III and VI. In the human cortex, the terminal field in layer I is even denser than in rat cortex; there are also labelled cell bodies in the subcortical white matter[17,22]. Human cortex, compared with rat cortex, has a much greater degree of regional variation in density of somatostatin-positive staining. The cingulate, temporal and frontal cortices have the densest somatostatin-positive terminal arborization of all neocortical regions; parietal cortex has an intermediate density and the primary visual cortex has the least dense innervation.

The general regional and laminar patterns of somatostatin immunoreactivity for terminals and cell bodies in Alzheimer cortices were similar to those in postmortem samples from normal elderly patients with no clinical or autopsy signs of Alzheimer's disease. All Alzheimer specimens, however, contained striking evidence of direct pathological involvement of somatostatin-containing cortical neurones. Swollen bulbous profiles interpreted as degenerating somatostatin-positive fibres (Figs 1, 2*a*, *b*), were readily apparent. With combined fluorescence bright-field optics, immunohistochemical material counterstained with thioflavin-S further revealed that these bulbous, clustered, swollen profiles were associated often with amyloid-containing neuritic plaques. Most of the plaques containing somatostatin-immunoreactive material appeared to be relatively 'immature', with no clear amyloid core. However, 'mature' plaques with a clear amyloid core and apparently degenerating somatostatin-positive profiles at the core perimeter were also observed. In addition, non-bulbous, somatostatin-positive fibres with a normal varicose appearance were seen often to surround or end just inside the periphery of mature plaques. Depending on the region and layer examined, 30–50% of the definitive early and mature plaques contained some somatostatin immunoreactivity. This percentage is surprisingly high for co-localizations based on single sections of plaques. Reconstruction of the entire three-dimensional extent of plaques would probably reveal an even higher proportion. Moreover, compared with perfused monkey cortex, only a fraction of somatostatin profiles are revealed in postmortem Alzheimer's material. Finally, despite lacking thioflavin staining for amyloid, other presumptively degenerating bulbous somatostatin-immunoreactive profiles could represent a stage of plaque formation where amyloid deposition is minimal, as in primitive plaques[23,24]. Indeed, some deteriorating somatostatin-immunoreactive profiles resemble clearly the primitive plaque of classical neuropathology (see Fig. 2*c*). Profiles similar to those seen in Alzheimer cases (Fig. 2) were seen rarely in normal aged cortices. Future experiments will aim at determining whether or not a given plaque contains multiple neurotransmitter markers.

The laminar and regional distributions of Bielschowsky-stained and thioflavin-positive plaques in Alzheimer material bear a striking similarity to distributions of somatostatin-positive terminals in normal human and animal material[17]. Most of the plaques in Alzheimer's specimens, like somatostatin-positive terminals in normal specimens, are in the supragranular layers, particularly layer III, with layer V having intermediate density and layer IV the lowest density of plaques and somatostatin innervation. Most of the somatostatin-positive plaque complexes (see Figs 1, 2*a*, *b*) are in layer III, where neurone loss in Alzheimer's disease is maximal[25] and most of the cortico-cortical association systems originate. In addition, plaque density in Alzheimer's material and somatostatin-terminal density in normal material show an almost identical regional profile: cingulate, frontal and temporal regions have the highest density of plaques and somatostatin-immunoreactive terminals, parietal cortex has an intermediate density of both and the primary visual cortex has the lowest of both. Thus, the limbic and association areas of the cortex, which are dominated by cortico-cortical association systems rather than thalamo-cortical connections, are the areas with the highest plaque density[26] and somatostatin

innervation. Collectively, these laminar and regional data suggest that plaque formation and somatostatin innervation are linked to similar elements of cortical circuitry. Plaques may arise from the degeneration of a complex of the postsynaptic neurites of large layer III or V projection neurones which form the cortical association systems[27] and their associated inputs containing neurotransmitters such as acetylcholine, noradrenaline, somatostatin or substance P[28].

The combined neurochemical and neuropathological findings in Alzheimer specimens lead to the hypothesis that functional deficits may result from the breakdown of both neocortical association systems and divergent, global cortical afferents such as the cholinergic projection from the nucleus basalis and the noradrenergic projection from the nucleus locus coeruleus. Whether the degeneration of these ascending systems is primary or secondary to a primary intra-cortical pathology, the breakdown of these global afferent systems plus a breakdown of the cortico-cortical associational systems could give rise to profound loss in attention, arousal and intellectual function, with minimal effects on specific sensory or motor performance. In this view, Alzheimer's disease could represent a dissociation of the various components of those cortical functions subserved by distributed systems[29]. The multiple transmitter systems involved in the pathological process suggest that no single transmitter can provide a therapeutic strategy and that the nature of the primary pathological insult remains the main question.

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1. Katzman, R. *Archs Neurol.* **33**, 217–218 (1976).
2. Terry, R. D. & Davies, P. *A. Rev. Neurosci.* **3**, 77–95 (1980).
3. Blessed, G., Tomlinson, B. E. & Roth, M. Brit. *J. Psychol.* **114**, 797–811 (1968).
4. Roth, M., Tomlinson, B. E. & Blessed, G. *Proc. R. Soc. Med.* **60**, 14–18 (1967).
5. Rossor, M. N. *Lancet* **ii**, 200 (1982).
6. Rossor, M. N., Iversen, L. L., Reynolds, G. P., Mountjoy, C. Q. & Roth, M. *Br. Med. J.* **288**, 961 (1984).
7. Davies, P. & Maloney, A. J. R. *Lancet* **ii**, 1403 (1976).
8. Coyle, J. T., Price, D. L. & DeLong, M. R. *Science* **219**, 1184–1190 (1983).
9. Cuello, A. C. & Sofroniew, M. V. *Trends Neurosci.* **7**, 74–78 (1984).
10. Whitehouse, P. J. *et al. Science* **215**, 1237–1239 (1982).
11. Struble, R. G., Cork, L. C., Whitehouse, P. J. & Price, D. L. *Science* **216**, 413–415 (1982).
12. Adolfsson, R., Gottfries, C. G., Roos, B. E. & Winblad, B. *Br. J. Psychiat.* **135**, 216 (1979).
13. Bondareff, W., Mountjoy, C. Q. & Roth, M. *Neurology* **32**, 164 (1982).
14. Iversen, L. L. *et al. Neurosci. Lett.* **39**, 95 (1983).
15. Davies, P., Katzman, R. & Terry, R. D. *Nature* **288**, 279 (1980).
16. Morrison, J. H., Benoit, R., Magistretti, P. J. & Bloom, F. E. *Brain Res.* **262**, 344–351 (1983).
17. Morrison, J. H., Rogers, J., Scherr, S. H., Benoit, R. & De Girolami, U. *Soc. Neurosci. Abstr.* **10**, 894 (1984).
18. Gottfries, C. *et al. Neurobiol. Aging* **4**, 261–272 (1983).
19. Rogers, J. & Bloom, F. E. in *Handbook of the Biology of Aging* (eds Finch, C. E. & Schneider, E.) (Van Nostrand Reinhold, New York, in the press).
20. Benoit, R., Ling, N., Alford, B. & Guillemin, R. *Biochem. biophys. Res. Commun.* **107**, 944–950 (1982).
21. Schwartz, P. *Gerontologia* **18**, 321 (1972).
22. Sorenson, K. V. *Neuroscience* **7**, 1227–1232 (1982).
23. Morimatsu, M., Hirai, S., Muramatsu, A. & Yoshikawa, M. *J. Am. Geriat. Soc.* **23**, 390–395 (1975).
24. Dayan, A. D. *Acta neuropath.* **16**, 85–95 (1970).
25. Terry, R. D., Peck, A., DeTeresa, R., Schechter, R. & Horoupian, D. S. *A. Neurol.* **10**, 184–192 (1981).
26. Rogers, J. & Morrison, J. H. *J. Neurosci.* (submitted).
27. Jones, E. G. in *The Organization of the Cerebral Cortex* (eds Schmitt, F. O., Worden, F. G., Adelman, F. & Dennis, S. G.) 199–235 (MIT, Cambridge, 1981).
28. Armstrong, D. M., Shields, D. & Terry, R. D. *Soc. Neurosci. Abstr.* **10**, 271 (1984).
29. Mountcastle, V. M. in *The Neurosciences—4th Study Program* (eds Schmitt, F. O. & Worden, F. G.) 21–42 (MIT, Cambridge, 1979).

Location of neuronal tangles in somatostatin neurones in Alzheimer's disease

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Senile dementia of the Alzheimer type is a chronic, progressive neuropsychiatric condition characterized clinically by global intellectual impairment[1] and neuropathologically by the presence of numerous argyrophilic plaques and tangles[2–4]. Neurochemical investigations have established loss of the cholinergic[5,6] and aminergic[7,8] projections to the cerebral cortex and a loss of the content of somatostatin[9–12], with preservation of cholecystokinin[11,13] and vasoactive intestinal polypeptide[11,14], neuropeptides also located in cells intrinsic to the cortex. We describe here the relationship between cortical somatostatin immunoreactivity and the plaques and tangles of diseased tissue by immunocytochemical and silver impregnation techniques on paraffin-embedded tissue. In sections of Alzheimer's tissue, cortical somatostatin-immunoreactive perikarya exhibited morphological changes consistent with neuronal degeneration. Silver-stained material immunostained subsequently showed that many neurones containing tangles were also somatostatin positive. No such co-localization was observed using antisera to other neuropeptides. Our findings indicate that a subclass of somatostatin-positive neurones are affected selectively in Alzheimer's disease and that these neurones also contain neuronal tangles. Thus, destruction of somatostatin-containing neurones is an early and perhaps critical event in the disease process.

Brains were obtained postmortem from six controls (4 males, 2 females, mean age 76 yr, range 58–87 yr) and 15 clinically and neuropathologically defined cases of Alzheimer's (5 male, 10 female, mean age 77 yr, range 57–91 yr). Brains were fixed routinely in 4% formalin and blocks of tissue which included the left temporal lobe structures (temporal cortex, hippocampus, amygdala) were taken and paraffin embedded, then 20-μm coronal sections cut for histological examination. Plaques and tangles were demonstrated using a modified Palmgren's method[15], somatostatin and other neuropeptides by immunocytochemical techniques (see Fig. 1 legend for details).

In control tissue, somatostatin immunoreactive neurones were numerous in temporal and entorhinal cortex and parahippocampal gyrus (Fig. 1*a*). The neurones were localized in cortical layers II–III and V–VI and were predominantly of the non-pyramidal type (multipolar and bitufted; see Fig. 1*b*), showing a range of morphological diversity. Numerous somatostatin fibres were also observed running radially with some lateral branching towards the pial surface and down to the subcortical white matter. Many somatostatin-positive neurones were found adjacent to the subcortical white matter with their dendritic fields oriented parallel to it; fibres from these cells penetrated into the white matter, possibly to form part of an intracortical association system. Somatostatin-positive neurones and fibres were also observed in the subiculum, pro-subiculum, hippocampal CA1–4 (stratum oriens and pyrimidale), the hilus of the dentate gyrus and the latero-basal, central, medial and cortical nuclei of the amygdala. Large numbers of multipolar (Golgi type II) neurones and fibres were also observed in the caudate nucleus and putamen (Fig. 1*c*).

On immunohistochemical examination, Alzheimer's cases showed marked decreases in somatostatin content. In the superficial layers (II and III) of the temporal cortex, parahippocampal gyrus and uncus, both neuronal and fibre staining was depleted substantially; some regions showed no observable immunoreactive neurones and had a concomitant decrease in local fibre innervation. In addition to the decreases in immunoreactive material, many somatostatin-positive cells (particularly in deeper cortical layers) were observed which showed morphological changes consistent with neuronal degeneration and

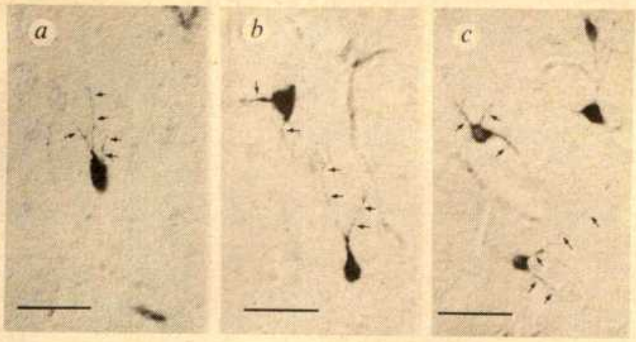


Fig. 1 Somatostatin-28(1-14 Tyr)-immunoreactive neurones in control tissue. *a*, Typical bitufted cell-type observed frequently in cortical layers II-V with dendrites oriented towards the pial surface (arrows). Different morphological subtypes of neurones are seen in the cortex. *b*, Multipolar cell with laterally extending dendrites (arrowed) in close proximity to a bitufted neurone with radially extending dendrites (arrowed) in layer III of the cortex. *c*, Example of the medium-sized aspiny (Golgi type II) somatostatin-immunoreactive interneurones found throughout the caudate. Each cell has two to four processes (arrowed) which curve as they course away from the cell body. Scale bars, 50 μm.

Methods. Sections used for the co-localization of tangles and somatostatin immunoreactivity were processed first for silver impregnation, rinsed in phosphate buffer and processed subsequently for immunocytochemistry. For silver impregnation, sections were processed according to the protocol of Cross[15]. For immunocytochemistry, 20-μm sections of tissue were incubated overnight with specific antisera raised to: somatostatin-28(1-14 Tyr) (RIA), dilution 1/1,000: somatostatin-14 (ref. 16), dilution 1/2,000; vasoactive intestinal polypeptide, dilution 1/10,000 (ref. 19); cholecystokinin-octapeptide, dilution 1/500 (ref. 19) and neuropeptide Y, dilution 1/1,000 (ref. 17) according to a previously described protocol for the peroxidase/anti-peroxidase method[16]. Pre-absorption of the diluted somatostatin-28(1-14 Tyr) antiserum with 50 μg somatostatin-28(1-14 Tyr) abolished all specific staining; pre-absorption of the antibody with other peptides had no effect. Specificity details of the other antisera used have been given elsewhere[16,17]. The antiserum raised to somatostatin-28(1-14 Tyr) gave much more intense staining of cell bodies, dendrites and fibres than the antiserum raised to somatostatin-14 (in agreement with the reports of Morrison *et al.*[38] for the rat brain). Therefore, the data reported here are obtained using the somatostatin-28(1-14 Tyr) antiserum.

analogous to those obtained in silver-impregnated sections (Fig. 2); these degenerate morphological profiles were present in hippocampal areas (subiculum, CA1 and hilus of dentate gyrus) and the amygdala (primarily medial and cortical nuclei). Conversely, somatostatin immunoreactivity in the basal ganglia (caudate nucleus and putamen) was unchanged, with normal degrees of fibre innervation and no evidence of neuronal degeneration. In silver-impregnated sections which were subsequently immunostained, neurones with argyrophilic tangles were often somatostatin-positive (Fig. 3). These silver/somatostatin-positive structures (SSPS) were observed in the hippocampus (Fig. 3*a*, *c*) cortical areas (layers II and III, less often in layers V and VI; Fig. 3*b*) and amygdala. In all cases, careful scanning revealed somatostatin-positive neurones which were not degenerate or impregnated with silver (sometimes in the middle of clusters of SSPS) and tangles unstained for somatostatin (Fig. 3*c*). No specific relationship was discernible between plaques and somatostatin-positive structures. Occasionally some plaques seemed to be associated closely with somatostatin-positive fibres.

To establish the specificity of the relationship between somatostatin-positive cells and tangles, additional sections were processed for immunocytochemistry or for silver impregnation followed by immunocytochemistry using specific antibodies to cholecystokinin-octapeptide, vasoactive intestinal polypeptide[16] and neuropeptide Y[17] (all present in intrinsic cortical neurones). Fibres containing these peptides were associated with plaques. None of these peptides was decreased in Alzheimer's disease, co-localized with neuronal tangles or displayed appreciable

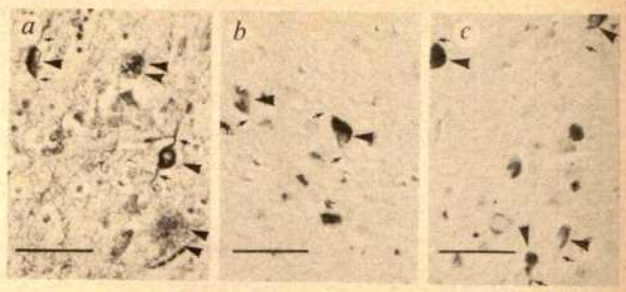


Fig. 2 *a*, Silver-impregnated section of temporal cortex showing typical tangles (single arrowhead) and plaques (double arrowheads) in a case of Alzheimer's disease. Note how dendrites of tangles are thickened and degenerate. *b*, Degenerate neuronal profiles of somatostatin-28-immunoreactive neurones in temporal cortex (layer III) of the Alzheimer's case (arrows). Note degenerating dendrites with almost total absence of staining (small arrows). *c*, Degenerate somatostatin-28-positive neurones in subiculum of hippocampus (arrows). Note presence of degenerating dendrites (small arrows). Scale bars, 50 μm.

numbers of conspicuously abnormal morphological profiles (Fig. 3*d*), with one partial exception. In the most severely affected cases (judged by neuropathological criteria), some neuropeptide Y-containing neurones in deeper cortical layers were degenerate and co-localized with neuronal tangles. In some cortical and subcortical neurones, neuropeptide Y is colocalized with somatostatin[18,19]. However, neuropeptide Y content is largely unchanged in Alzheimer's disease[23]; thus, the observed degeneration may be a late change. These results agree with radioimmunoassay studies demonstrating (1) that somatostatin concentrations are higher in the temporal than other cortices and (2) that although somatostatin is decreased in Alzheimer's disease, other peptides (including neuropeptide Y) are relatively unaffected[11,13,14,20].

Both plaques and tangles are related quantitatively to dementia scores[21-23]; both indices of pathology are also associated with the decreases in choline acetyltransferase (CAT) in the frontal and temporal cortex[21-24]. Indeed, reports of CAT and acetylcholinesterase staining in plaques has led to the proposal that plaques are composed of (at least in part) degenerating cholinergic terminals[25]. The relationship between plaques and CAT has prompted studies on the basal nucleus of Meynert—the source of almost all the cortical cholinergic innervation. This nucleus shows considerable cell loss and/or atrophy in Alzheimer's disease, as do the cell bodies of origin of the monoaminergic projections to the cerebral cortex, for example, the locus coeruleus[26-28]. These findings have prompted speculations that Alzheimer's is a disease of the isodendritic core[29], the primary lesion being subcortical (and cholinergic) and the cortical pathology resulting from transynaptic degeneration caused by the loss of ascending fibres. Therefore, the plaques and tangles are viewed "as a secondary response to the loss of ascending inputs"[29]. If this were correct, a loss of neurones in the basal nucleus of Meynert would lead to a drop in cortical CAT and somatostatin. In rats, lesions to the basal nucleus of Meynert do lead to cholinergic deficits but cortical somatostatin levels remain unchanged[30]. Conversely, lesions of the cortex (which produce somatostatin losses along with losses of other intrinsic transmitters in both rats and man) result in retrograde degeneration of cholinergic cells in the basal nucleus of Meynert similar to that seen in Alzheimer's disease[31,32]. These reports and the correlations between cortical pathology and symptoms imply that the cortex is the seat of pathology and that, in particular, the temporal cortex is the main focus of the disease process. The findings reported here that some plaques (which may include degenerating cholinergic terminals) contain somatostatin fibres (confirming a previous observation)[33] and reports that plaques also contain dendrites and axons of diverse local neurones and perhaps glial elements[34] indicate that plaques may be a neurochemically nonspecific consequence of the disease process.

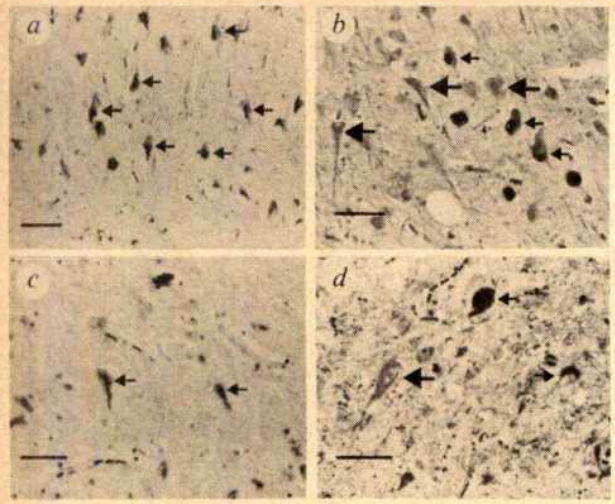


Fig. 3 Silver-stained sections (in which tangles stain black) were immunostained subsequently to detect the presence of somatostatin immunoreactivity (dark precipitate). In these sections many silver/somatostatin-positive structures (SSPS, dark staining) are seen in temporal regions in Alzheimer's cases. *a*, The subiculum of the hippocampus showed numerous SSPS (arrowed) as did *b*, neurones (arrowed) in layers II, III and V of the parahippocampal gyrus. *c*, The CA1 pyramidal region of the hippocampus also contained many SSPS (small arrows); it was observed often that even in regions which showed numerous SSPS, apparently normal somatostatin-28-positive neurones could still be observed (large arrows). *d*, Staining with antiserum to other peptides, for example vasoactive intestinal peptide, demonstrated that in the cortex, tangles (small arrows) did not occur in vasoactive intestinal peptide-immunoreactive neurones (large arrow). Scale bars, 50 μm.

The findings demonstrate a strong link between the formation of neuronal tangles (their presence highly correlated with the symptoms of dementia) and the decrease of immunoreactivity to somatostatin (the only intrinsic cortical transmitter known to be affected) in the temporal cortex (pathologically the area most affected). We suggest that somatostatin neurones in the temporal cortex are a primary focus for the disease process and that the loss of somatostatin content is an early event in the production of symptoms and the subsequent (or concomitant) cortical atrophy. The cholinergic deficit and atrophy of the basal nucleus of Meynert may be a secondary consequence of the loss of cortical somatostatin neurones from a region which provides the major input to the basal nucleus[35]. The reason for the selectivity of the disease process is unknown but presumably is related to the physiological properties of susceptible somatostatin neurones in the cortex. The structure of somatostatin itself (particulary the helical fibril-forming noncyclic form[36]) or the recently described somatostatin gene[37] may be relevant.

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1. Katzman, R. (ed.) *Banbury Report* Vol. 15 (Cold Spring Harbor Laboratory, New York, 1983.)
2. Corsellis, J. A. N. in *Greenfields Neuropathology* 2nd edn (eds Blackwood, W. & Corsellis, J. A. N.) 798–848 (Arnold, London, 1976).
3. Hooper, M. W. & Vogel, S. *Am. J. Path.* **85,** 1–13 (1976).
4. Brun, A. & Englund, E. *Histopathology* **5,** 549–564 (1981).
5. Bowen, D. M., Smith, C. B., White, P. & Davison, A. N. *Brain* **99,** 457–496 (1976).
6. Davies, P. & Maloney, A. J. *Lancet* **ii,** 1430 (1976).
7. Cross, A. J. *et al. Br. med. J.* **282,** 93–94 (1981).
8. Adolfsson, R., Gottfries, C. G., Roos, B. E. & Winblad, B. *Br. J. Psychol.* **135,** 216–223 (1979).
9. Davies, P. & Terry, R. D. *Neurobiol. Aging* **2,** 9–14 (1981).
10. Rossor, M., Emson, P. C., Mountjoy, C. G., Roth, S. M. & Iversen, L. L. *Neurosci. Lett.* **20,** 373–377 (1980).
11. Ferrier, I. N. *et al. J. neurol. Sci.* **62,** 159–170 (1983).
12. Perry, R. H. *et al. J. neurol. Sci.* **51,** 465–472 (1981).
13. Rossor, M. N. *et al. Life Sci.* **29,** 405–410 (1981).
14. Rossor, M. N. *et al. Brain Res.* **201,** 249–253 (1980).
15. Cross, R. B. *Med. Lab. Sci* **39,** 67–69 (1982).
16. Roberts, G. W., Woodhams, P. L., Polak, J. M. & Crow, T. J. *Neuroscience* **7,** 99–131 (1982).
17. Adrian, T. E. *et al. Nature* **306,** 584–586.
18. Vincent, S. R. *et al. Nature* **298,** 65–67 (1982).
19. Vincent, S. R. *et al. Neuroscience* **7,** 439–446 (1982).
20. Allen, J. M. *et al. J. neurol. Sci.* **64,** 325–331 (1984).
21. Perry, R. H. *et al. Br. med. J.* **II,** 1457–1459 (1978).
22. Wilcock, G. K. & Esiri, M. *J. Neurol. Sci.* **57,** 407–417 (1982).
23. Wilcock, G. K. *Gerontology* **29,** 320–324 (1983).
24. Mountjoy, C. Q., Rossor, M. N., Iversen, L. L. & Roth, M. *Brain* **107,** 507–515 (1984).
25. Tomlinson, B. E. *Psychol. Med.* **12,** 449–459 (1982).
26. Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, T. T. & Delong, M. *Ann. Neurol.* **10,** 122–126 (1981).
27. Perry, R. H. *et al. Neurosci. Lett.* **33,** 311–315 (1982).
28. Tomlinson, B. E., Irving, D. & Blessed, G. *J. neurol. Sci.* **49,** 419–428 (1981).
29. Rossor, M. *Br. med. J.* **283,** 1588–1590 (1981).
30. McKinney, M., Davies, P. & Coyle, J. T. *Brain Res.* **243,** 169–172 (1982).
31. Sofroniew, M., Pearson, R. C. A., Eckenstein, F., Cuello, A. C. & Powell, T. P. S. *Brain Res.* **289,** 370–374 (1983).
32. Pearson, R. C. A., Gatter, K. C. & Powell, T. P. S. *Brain Res.* **261,** 321–326 (1983).
33. Perry, R. H. *et al.* in *Banbury Report* Vol. 15 (ed. Katzman, R.) 351–361 (Cold Spring Harbor, Laboratory, New York, 1983.)
34. Probst, A., Basler, V., Bron, B. & Ulrich, J. *Brain Res.* **268,** 249–254 (1983).
35. Mesulam, M. & Mufson, E. J. *Brain* **107,** 253–274 (1984).
36. Oakley, A. E., Perry, R. H., Candy, J. M. & Perry, E. K. *Neuropeptides* **2,** 1 (1981).
37. Shen, L. P. & Rutter, W. J. *Science* **224,** 168–171 (1984).
38. Morrison, J. H., Benoit, R., Magistretti, P. J. & Bloom, F. E. *Brain Res.* **262,** 344–351 (1983).

A functional correlate for the dihydropyridine binding site in rat brain

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Calcium channels, controlling the influx of extracellular Ca^{2+} and hence neurotransmitter release, exist in the brain[1]. However, drugs classed as calcium antagonists and which inhibit Ca^{2+} entry through voltage-activated Ca^{2+} channels in heart and smooth muscle[2], seem not to affect any aspect of neuronal function in the brain at pharmacologically relevant concentrations[3–6]. Yet the dihydropyridine calcium antagonists (for example, nitrendipine) bind stereospecifically with high affinity to a recognition site on brain-cell membranes thought to represent the Ca^{2+} channel[4,7,8] and consequently, the physiological relevance of these sites has been questioned[9]. However, activation of voltage-dependent Ca^{2+} channels can increase cytoplasmic Ca^{2+} and neurotransmitter release in neuronal tissue[10]. We show here that Bay K 8644, a dihydrophyridine Ca^{2+}-channel activator[11,12], can augment K^+-stimulated release of serotonin from rat frontal cortex slices and that these effects can be antagonized by low concentrations of calcium antagonists. As ^{3}H-dihydropyridine binding to cortical membrane preparations resembles the binding in heart and smooth muscle where there are good functional correlates[8,13] we conclude that the dihydropyridine binding sites in the brain represent functional Ca^{2+} channels that can be unmasked under certain circumstances.

We have used K^+-induced depolarization of rat cortical slices to stimulate Ca^{2+} entry and evoke ^{3}H-serotonin release from the cortical serotonergic neurones as a functional correlate of the dihydropyridine binding site in brain tissue. Slices of the rat frontal cortex were incubated in Krebs medium with ^{3}H-serotonin to label specifically the neurotransmitter stores in serotonergic nerve terminals. The slices were then superfused with physiological medium and exposed to two stimuli of Krebs medium containing elevated K^+ (25 mM). The first stimulus (S_1) occurred 38–42 min after the start of the superfusion and the second (S_2) at 78–82 min. ^{3}H-serotonin release is coupled closely to Ca^{2+} entry[14] and K^+-evoked release in our experiments was entirely Ca^{2+} dependent. Drugs (when present) were added at $t = 58$ min and their effects quantified by the standard method of comparing the efflux of radioactivity evoked by the first K^+ stimulus with that evoked by the second K^+ stimulus (S_2/S_1 ratio). Effects on basal release rates were quantified by measuring the ratio of basal outflow immediately before S_1 compared with that immediately before S_2.

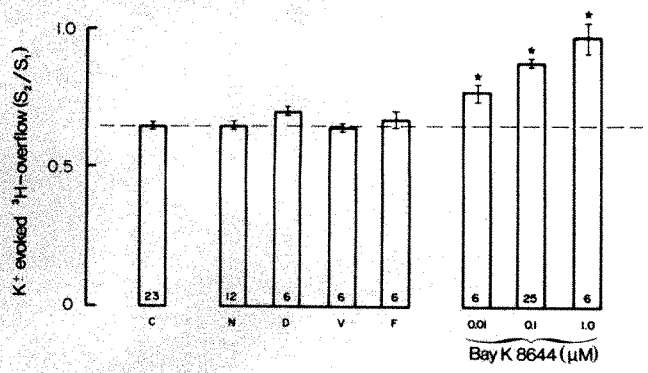


Fig. 1 Effect of calcium antagonists and Bay K 8644 on K^+-evoked release of ^{3}H-serotonin from rat frontal cortex slices. C, control; N, nifedipine (1 μM); D, diltiazem (1 μM); V, verapamil (1 μM); F, flunarizine (1 μM).

Methods. The frontal cortices from two male Sprague–Dawley rats (200–250 g) were dissected rapidly and chopped in two directions at 250 μm on a McIlwain brain-tissue chopper. The slices (total tissue weight 200–250 mg) were incubated for 15 min at 37 °C in 5 ml Krebs/Ringer-bicarbonate solution (composition (mM): NaCl 135, KCl 5, $NaHCO_3$ 25, $MgSO_4$ 1, KH_2PO_4 1.25, $CaCl_2$ 2, glucose 10; gassed with 95% O_2 and 5% CO_2) containing 0.1 μM ^{3}H-serotonin (specific activity 28.3 Ci mmol^{-1}) and 10 μM pargyline. After three washes with 5 ml Krebs buffer, 50-μl aliquots of the slices (~20 mg tissue) were transferred to each chamber (100 μl vol) of a superfusion apparatus and superfused at a rate of 0.38 ml min^{-1} with Krebs buffer containing 3.2 μM paroxetine to prevent the re-uptake of serotonin into the nerve endings. After 30 min superfusion ($t = 30$ min), 18 successive 4-min fractions were collected ($t = 30$ to $t = 102$ min). The slices were exposed to two separate 4-min stimuli by Krebs buffer containing 25 mM K^+ ions (isosmolar replacement of NaCl by KCl) starting at $t = 38$ and 78 min. Drugs were added to the superfusate at $t = 58$ min and were present throughout the rest of the superfusion period. After the radioactivity in the fractions and that remaining in the tissue slices had been determined by liquid scintillation counting, the fractional efflux rate of each fraction was determined. The magnitude of the efflux evoked by the first stimulus (S_1) was then compared with that evoked by the second stimulus (S_2) by measuring the additional efflux evoked by the 25 mM K^+ Krebs during the time period $t = 38$–58 min (S_1) and 78–98 min (S_2). For the purpose of this calculation, a linear decline in the efflux of ^{3}H was assumed between $t = 38$–62 min and $t = 78$–102 min for the calculation of S_1 and S_2 respectively. At $t = 42$ min, control basal release rates in the absence of added drugs were 0.71 ± 0.02% tissue stores per min (mean ± s.e.m., $n = 29$). The additional release evoked by the first 4-min exposure to 25 mM K^+ Krebs (S_1) was 10.20 ± 0.48% tissue stores ($n = 29$) and to the second exposure (S_2) 6.72 ± 0.36% tissue stores ($n = 29$). Drug effects were quantified by calculating the S_2/S_1 ratio. Results are mean ± s.e.m. Numbers in the histograms indicate the number of separate experiments. Statistical significance of drug effects compared with controls was assessed using a two-tailed Mann–Whitney U test ($^*P < 0.05$).

Although the K^+-evoked ^{3}H-serotonin release was entirely Ca^{2+} dependent, a range of calcium antagonists with disparate structures and different sites of action did not affect ^{3}H-serotonin release at concentrations which cause considerable antagonism at Ca^{2+} channels in smooth muscle preparations[15] (Fig. 1). This lack of effect on K^+-evoked release was accompanied by a small but non-significant enhancement of basal ^{3}H release, consistent with the negligible effects of calcium antagonists on neurotransmitter release reported previously[5,6].

Bay K 8644 both activates Ca^{2+} channels[11,12] and increases rather than antagonizes Ca^{2+} entry[16]. It binds to the dihydropyridine recognition site and, in the rat cortex, displaces ^{3}H-nimodipine binding with a K_i of 30 nM (ref. 17). Basal release of ^{3}H-serotonin from rat frontal cortex slices was not modified significantly by Bay K 8644 (0.01–1 μM), but K^+-evoked serotonin release was augmented in a concentration-dependent manner (0.01–1 μM; Fig. 1). Thus, in the presence of 1 μM Bay 8644, the S_2/S_1 ratio was 0.97 ± 0.06 ($n = 6$), an

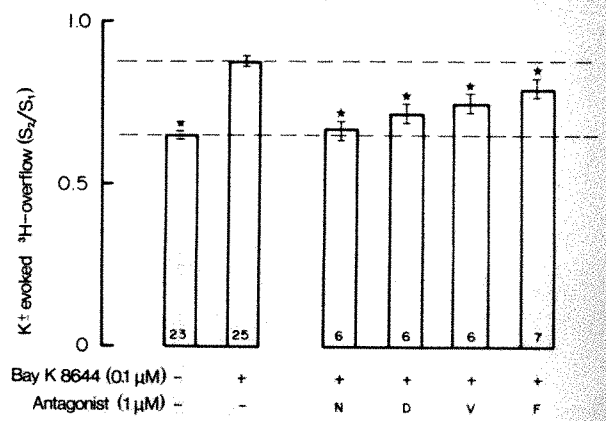


Fig. 2 Effect of calcium antagonists on the augmentation of K^+-evoked release of ^{3}H-serotonin from rat frontal cortex slices by Bay K 8644. N, nifedipine; D, diltiazem; V, verapamil; F, flunarizine. Experiments were performed as described in Fig. 1 legend. Bay K 8644 (0.1 μM) and the relevant antagonist (1 μM) were added to the superfusion medium at $t = 58$ min and were present throughout the rest of the experiment. Results are mean ± s.e.m. Numbers in the histograms indicate the number of separate experiments. Statistical significance of effects, compared with Bay K 8644 alone, were assessed using a two-tailed Mann–Whitney U test ($^*P < 0.05$).

increase of 49% above the control value ($S_2/S_1 = 0.65 \pm 0.01$; $n = 23$). In this respect the effects of Bay K 8644 on ^{3}H-serotonin release resemble the effects on K^+-induced contractions in smooth muscle, where the compound increases tone only when the Ca^{2+} channels are stimulated by K^+-depolarization[12]. Furthermore, the concentrations of Bay K 8644 required to augment ^{3}H-serotonin release were similar to those required to displace ^{3}H-nimodipine binding in this tissue[17]. A submaximal concentration of Bay K 8644 (0.1 μM), which increased the S_2/S_1 ratio by 35% (S_2/S_1 ratio in the presence of 0.1 μM Bay K 8644 = 0.88 ± 0.02; $n = 25$), was chosen to investigate antagonist interactions.

The concentrations of the calcium antagonists which had no effect on K^+-evoked ^{3}H-serotonin release attenuated the stimulant effects of Bay K 8644 (Fig. 2). These concentrations have been shown to block Ca^{2+}-induced contractions in smooth muscle in the presence of Bay K 8644 (ref. 18); however, these concentrations are sufficiently low to exclude effects on ion channels other than the Ca^{2+} channel[2].

This interpretation is supported by experiments showing that the augmentation of K^+-evoked release induced by the serotonin autoreceptor antagonist methiothepin was not attenuated by nifedipine. Thus, methiothepin (0.3 μM added after S_1) increased the S_2/S_1 ratio to 0.94 ± 0.05 (mean ± s.e.m.; $n = 6$) and a similar agumentation was also seen if nifedipine (1 μM) was added to the superfusate at the same time as the methiothepin (S_2/S_1 ratio 1.02 ± 0.06; not significant against methiothepin alone; $n = 6$). The stimulant effects of Bay K 8644 can therefore be attributed to stimulation of voltage-dependent Ca^{2+} channels which, in the presence of this activator, resemble those in smooth muscle cells[18] or in cells derived from the adrenal glands[19–22] in their susceptibility to organic calcium antagonists. Previous studies using tissues derived from sources other than the brain give conflicting evidence about the effects of Bay K 8644 on transmitter release[15,22,23]. The reasons for these differences are not yet understood, but may reside in the nature of the different stimuli used to release the transmitters. Thus, Bay K 8644 can augment K^+-evoked release of catecholamines from chromaffin tissue[19,22] but not noradrenaline release from sympathetic neurones evoked by supramaximal electrical stimulation[23]. We have observed that Bay K 8644 (0.1 μM) failed to augment K^+-evoked release of ^{3}H-serotonin from rat frontal cortex slices when the K^+ stimulus was increased to 50 mM (data not shown). We therefore consider that Bay K 8644 will only augment neurotransmitter release in conditions of submaximal stimulation. Nevertheless, our experiments have shown clearly that

Ca^{2+} channels in the brain differ from those in the heart and smooth muscle because they become sensitive to organic calcium antagonists only in the presence of Bay K 8644. One explanation for these apparent differences between central and peripheral Ca^{2+} channels may result from the recent discovery that Ca^{2+} channels comprise several subunits[24-26] differentially affected by organic calcium antagonists[27]. It is therefore possible that the subunits of the Ca^{2+} channel in nervous tissue are arranged differently from those in smooth muscle and the presence of Bay K 8644 is required to induce a conformational change, not only activating the channels but also conferring sensitivity to the antagonists. Such an effect could have important consequences if neuronal Ca^{2+} channels could be activated similarly in physiological or pathological conditions. Thus, calcium antagonists would have little effect on normal neuronal function but may cause marked inhibition of excess Ca^{2+} entry following certain stimuli. Such stimuli may occur in pathological conditions as the anoxia-induced noradrenaline release from nerves in the heart is susceptible to calcium antagonists[28].

Thus, dihydropyridines have a defined functional role in the control of neurotransmitter release, affecting release at similar concentrations to those at which they bind to neuronal tissues[17] and to those at which they affect smooth muscle cells[18]. The striking effects of Bay K 8644 in our experiments suggest that this Ca^{2+} channel activator will prove a useful tool with which to evaluate the functional effects of calcium antagonists in the brain.

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1. Nelson, M. T., French, R. J. & Krueger, B. K. *Nature* **308**, 77-80 (1984).
2. Fleckenstein, A. *Calcium Antagonism in Heart and Smooth Muscle* (Wiley, New York, 1983).
3. Daniell, L. C., Barr, E. M. & Leslie, S. W. *J. Neurochem.* **41**, 1455-1459 (1983).
4. Snyder, S. H. *Science* **224**, 22-31 (1984).
5. Miller, R. J. & Freedman, S. B. *Life Sci.* **34**, 1205-1221 (1984).
6. Starke, K., Späth, L. & Wichmann, T. *Naunyn-Schmiedebergs Archs Pharmak.* **325**, 124-130 (1984).
7. Glossmann, H., Ferry, D. R., Lübbecke, F., Mewes, R. & Hofmann, F. *Trends pharmac. Sci.* **3**, 431-437 (1982).
8. Janis, R. A. & Scriabine, A. *Biochem. Pharmac.* **32**, 3499-3507 (1983).
9. Triggle, D. J. *Trends pharmac. Sci.* **5**, 4-5 (1984).
10. Ashley, R. H., Brammer, M. J. & Marchbanks, R. *Biochem. J.* **219**, 149-158 (1984).
11. Schramm, M., Thomas, G., Towart, R. & Franckowiak, G. *Nature* **309**, 535-537 (1983).
12. Schramm, M., Thomas, G., Towart, R. & Franckowiak, G. *Arzneimittel-Forsch.* **33**, 1268-1272 (1983).
13. Bolger, G. T. *et al. J. Pharmac. exp. Ther.* **225**, 291-309 (1983).
14. Göthert, M. *Trends pharmac. Sci.* **3**, 437-440 (1982).
15. Spedding, M. *Naunyn Schmiedebergs Archs Pharmak.* **318**, 234-240 (1982).
16. Anderson, C. L., Scriabine, A. & Janis, R. A. *Fedn Proc.* **43**, 550 (1984).
17. Bellemann, P. *FEBS Lett.* **167**, 88-92 (1984).
18. Spedding, M. & Berg, C. *Naunyn-Schmiedebergs Archs Pharmak.* **328**, 69-75 (1984).
19. Albus, U., Habermann, E., Ferry, D. R. & Glossmann, H. *J. Neurochem.* **42**, 1186-1189 (1984).
20. Toll, L. *J. biol. Chem.* **257**, 13189-13192 (1982).
21. Takahashi, M. & Ogwa, A. *FEBS Lett.* **152**, 191-194 (1983).
22. Garcia, A. G. *et al. Nature* **309**, 69-71 (1984).
23. Docherty, J. R., Hyland, L. & Warnock, P. *B. J. Pharmac.* **82**, 213P (1984).
24. Venter, J. C. *et al. J. biol. Chem.* **258**, 9344-9348 (1983).
25. Ferry, D. R., Rombusch, M., Goll, A. & Glossmann, H. *FEBS Lett.* **169**, 112-119 (1984).
26. Glossmann, H. & Ferry, D. R. *Naunyn-Schmiedebergs Archs Pharmak.* **323**, 279-291 (1983).
27. Goll, A., Ferry, D. R. & Glossmann, H. *FEBS Lett.* **157**, 63-69 (1983).
28. Nayler, W. G. & Sturrock, W. J. *J. molec. cell. Cardiol.* **16**, 331-344 (1983).

Identification of a distinct synaptic glutamate receptor on horizontal cells in mudpuppy retina

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The separation of ON and OFF channels and the development of an antagonistic surround occur at the first synapse in the vertebrate retina[1-3]. This functional differentiation is mediated by the action of the photoreceptor neurotransmitter on the ON bipolar, OFF bipolar and horizontal cells, respectively. Glutamate mimics the action of the photoreceptor transmitter on all second-order neurones in fish[4,5], amphibian[6] and mammalian[7] retinas. The diversity of cellular responses produced by one neurotransmitter raises the possibility of multiple postsynaptic receptor–ionophore complexes. We reported previously that one glutamate analogue, 2-amino-4-phosphonobutyrate, reveals that the ON bipolar synaptic receptor is pharmacologically different from those of other second-order neurones[8,9]. The results presented here demonstrate that another glutamate analogue, D-*O*-phosphoserine, selectively antagonizes the synaptic responses of horizontal cells. Taken together, these findings indicate that there are three glutamate-like receptor subtypes in the outer retina and suggest a correlation between receptor subtype and the physiological properties of second-order neurones.

Experiments were performed on the superfused retina-eyecup preparation of the mudpuppy, *Necturus maculosus*. The hemisected eyecup was superfused with amphibian Ringer's solution (111 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM dextrose, 5 mM HEPES, *p*H 7.80) to which pharmacological agents could be added. Standard intracellular electrophysiological techniques were used to evaluate the effects of drugs on the light-driven synaptic responses of second-order neurones. A complete description of the methodology has been published previously[6,10].

Glutamate analogues such as kainic acid and quisqualic acid mimic the action of the photoreceptor neurotransmitter on all mudpuppy second-order neurones[6]. In the present experiments, we examined the effects of the D form of a glutamate analogue, *O*-phosphoserine, on exogenously applied glutamate analogues and on the endogenous photoreceptor neurotransmitter. When the retina was superfused with 5 mM D-*O*-phosphoserine, photoreceptor light responses were unchanged or only slightly enhanced. However, the drug had a selective effect on synaptic transmission between photoreceptors and second-order neurones. The photoreceptor neurotransmitter is released maximally in the dark, causing horizontal cells to be depolarized[11-13]. Figure 1*a* shows that D-*O*-phosphoserine at 2.5 mM hyperpolarized the 'dark' membrane potential of the horizontal cell and diminished the light response by 50%, consistent with a blocking of the photoreceptor neurotransmitter. Both small spot- and annulus-evoked responses were similarly affected, and the antagonism was dose-dependent over the range 1-10 mM. In contrast to this action of D-*O*-phosphoserine on horizontal cells, the direct synaptic communication between photoreceptors and bipolar cells was not diminished by the drug. Figure 1*b* shows that the 'centre' response of OFF bipolars to small-spot or annular stimulation remained essentially unaltered during a relatively prolonged application of 5 mM D-*O*-phosphoserine. However, the antagonistic surround response was blocked (see Fig. 1 legend). The surround response of bipolar cells is believed to result from horizontal cell feedback[14-19]. Therefore, the maintenance of the centre response during the loss of the surround response in the OFF bipolar is consistent with a selective blocking of the horizontal cells. In Fig. 1*c*, D-*O*-phosphoserine was applied to the retina while recording from an ON bipolar cell; as with the OFF bipolar, the drug did not reduce the centre response but the antagonistic surround response was suppressed.

To explore the pharmacology of D-*O*-phosphoserine, we compared its action with that of known excitatory amino-acid agon-

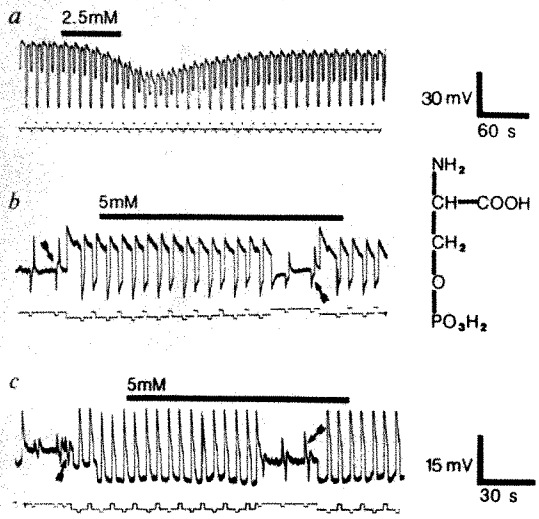


Fig. 1 Action of D-*O*-phosphoserine on the light responses of second-order neurones. *a*, 2.5 mM D-*O*-phosphoserine hyperpolarized and suppressed 50% of the synaptic responses of horizontal cells, while more prolonged application of twice this concentration did not diminish the centre responses of either OFF (*b*) or ON (*c*) bipolar cells. However, the sustained antagonistic surround response in bipolar cells, evoked by flashing an annulus during maintained small-spot illumination, was suppressed; this is shown by the reversal in polarity of the surround responses indicated by arrows before and during drug application. Bars above the traces indicate the duration of drug application; square pulses below the voltage traces mark the time of light stimuli. Upward pulses represent small spots (200 μm) and downward pulses mark annular stimulation (internal diameter 400 μm, external diameter greater than retinal diameter). Illumination, 5×10^{-9} W cm^{-2}. The lower calibration applies to *b* and *c*. The molecular formula for D-*O*-phosphoserine is shown at the right.

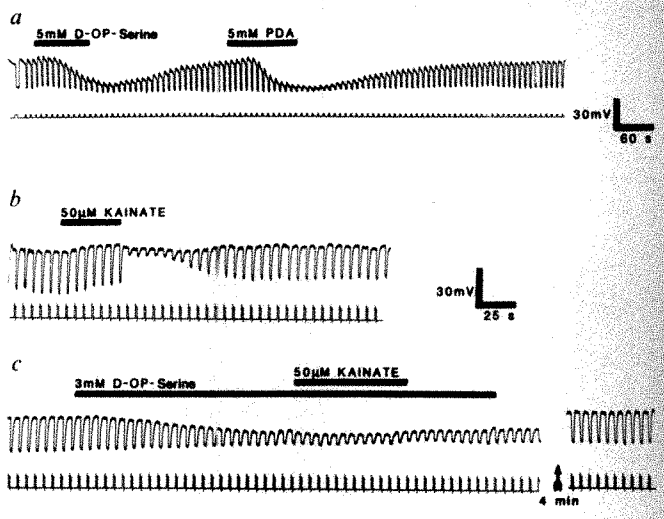


Fig. 2 *a*, Similarity of action of D-*O*-phosphoserine (D-OP-Serine) and *cis*-2,3-piperidine dicarboxylic acid (PDA; Cambridge Research Biochemicals), an excitatory amino-acid antagonist, on the light responses of a horizontal cell. The traces in *b* and *c*, from the same horizontal cell, show that D-*O*-phosphoserine suppresses the action of both kainic acid and the photoreceptor neurotransmitter. Diffuse light stimuli were used.

ists and antagonists. One antagonist that is effective on second-order neurones is *cis*-2,3-piperidine dicarboxylic acid (PDA), which blocks photoreceptor input to both OFF bipolars and horizontal cells[19]. At equivalent concentrations, D-*O*-phosphoserine and *cis*-2,3-piperidine dicarboxyclic acid had a similar effect on the synaptic responses of horizontal cells (Fig. 2*a*) although PDA was a slightly more potent antagonist.

The photoreceptor–horizontal cell synapse is mediated by a glutamate-preferring receptor and kainic acid mimics the action of the photoreceptor neurotransmitter[6]. Therefore, we evaluated the ability of D-*O*-phosphoserine to antagonize the effects of exogenously applied kainic acid. Figure 2*b* illustrates that a brief application of 50 μM kainic acid drives a horizontal cell towards the dark resting membrane potential and suppresses the light response, mimicking the endogenous transmitter. Figure 2*c* shows the same cell after recovery from the kainic acid-induced depolarization. D-*O*-phosphoserine at 3 mM produced a hyperpolarization and a reduction of the light response; in the continued presence of the drug, a more prolonged application of kainic acid had little effect on the neurone. γ-Aminobutyric acid (GABA) at 5 mM directly depolarizes mudpuppy horizontal cells to close to their dark membrane potential (M.S., unpublished observation); D-*O*-phosphoserine does not block this GABA-induced depolarization, indicating that the former is not acting by shunting horizontal cells towards an equilibrium potential below the dark membrane potential. These results suggest that D-*O*-phosphoserine suppresses the response of horizontal cells to light by blocking glutamate-like synaptic receptors.

Although it is not very potent, D-*O*-phosphoserine seems to possess a selectivity that may be useful as a pharmacological probe to identify and characterize excitatory amino-acid receptors. This ligand has also been reported to displace glutamate at some binding sites in rat forebrain[20]. The action of D-*O*-phosphoserine in the distal retina reveals a discrete glutamate receptor subtype at the horizontal cell synapse. We have reported previously that the ON bipolar cell possesses a synaptic glutamate receptor not found in other retinal neurones[9]. From these observations we conclude that there are three glutamate receptor subtypes in the outer plexiform layer and that each pharmacological subtype is found on a distinct physiological class of second-order neurone.

The reason for this receptor diversity and functional segregation is unclear; it may be related to receptor–ionophore coupling as it has been proposed that the synaptic potential of each class of second-order neurone is generated by a discrete ionic mechanism[21–23]. Another possible explanation, suggested by noise analysis in dogfish and turtle retinas, is that the kinetics of synaptic communication differs among the second-order neurones[24–26]. This may be an important coding mechanism for information transfer mediated by postsynaptic receptors. The differentiation of excitatory amino-acid receptors in the distal retina may elucidate further the role of receptors in synaptic communication.

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1. Werblin, F. & Dowling, J. E. *J. Neurophysiol.* **32**, 339–355 (1969).
2. Kaneko, A. *J. Physiol., Lond.* **207**, 622–633 (1970).
3. Burkhardt, D. A. *J. Physiol., Lond.* **236**, 593–610 (1974).
4. Murakami, M., Ohtsu, K. & Ohtsuka, T. *J. Physiol., Lond.* **227**, 889–913 (1972).
5. Murakami, M., Ohtsuka, T. & Shimazaki, H. *Vision Res.* **15**, 456–458 (1975).
6. Slaughter, M. M. & Miller, R. F. *J. Neurosci.* **3**, 1701–1711 (1983).
7. Bloomfield, S. A. & Dowling, J. E. *Soc. Neurosci. Abstr.* **8**, 131 (1982).
8. Slaughter, M. M. & Miller, R. F. *Science* **211**, 182–185 (1981).
9. Slaughter, M. M. & Miller, R. F. *J. Neurosci.* (in the press).
10. Miller, R. F. & Dacheux, R. F. *J. gen. Physiol.* **67**, 639–659 (1976).
11. Trifonov, J. A. *Biofizika* **13**, 809–814 (1968).
12. Dowling, J. E. & Ripps, H. *Nature* **242**, 101–103 (1973).
13. Dacheux, R. F. & Miller, R. F. *Science* **191**, 963–964 (1976).
14. Baylor, D. A., Fuortes, M. G. F. & O'Bryan, P. M. *J. Physiol., Lond.* **214**, 265–294 (1971).
15. Toyoda, J.-I. & Tonosaki, K. *Nature* **276**, 399–400 (1978).
16. O'Bryan, P. M. *J. Physiol., Lond.* **235**, 207–223 (1973).
17. Attwell, D., Werblin, F. S., Wilson, M. & Wu, S. *J. Physiol., Lond.* **336**, 313–333 (1983).
18. Skrzypek, J. & Werblin, F. *J. Neurophysiol.* **49**, 1007–1016 (1983).
19. Slaughter, M. M. & Miller, R. F. *Science* **219**, 1230–1232 (1978).
20. Foster, A. C., Fagg, G. E., Harris, E. W. & Cotman, C. W. *Brain Res.* **242**, 374–377 (1982).
21. Nelson, R. *J. Neurophysiol.* **36**, 519–535 (1973).
22. Saito, T., Kondo, H. & Toyoda, J. *J. gen. Physiol.* **73**, 73–90 (1979).
23. Saito, T. & Kaneko, A. *J. gen. Physiol.* **81**, 589–601 (1983).
24. Ashmore, J. F. & Copenhagen, D. R. *Nature* **288**, 84–86 (1980).
25. Ashmore, J. F. & Falk, G. *J. Physiol., Lond.* **300**, 115–150 (1980).
26. Copenhagen, D. R., Ashmore, J. F. & Schnapf, J. K. *Vision Res.* **23**, 363–369 (1983).

Expression of interleukin-2 receptors as a differentiation marker on intrathymic stem cells

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The thymus is regarded as the primary site for T-cell lymphopoiesis[1], but very little is known about the lineage interrelationships of cells within that organ. At least four subpopulations of mouse thymocytes can be defined on the basis of staining with monoclonal antibodies directed against the T-cell differentiation antigens Lyt-2 and L3T4 (ref. 2). Thus immunocompetent (medullary) thymocytes, like peripheral T cells, express either Lyt-2 (cytotoxic phenotype) or L3T4 (helper phenotype) but not both, whereas non-functional (cortical) thymocytes express both markers. In addition, a small subpopulation comprising 2–3% of cells in the thymus and expressing neither Lyt-2 nor L3T4 has recently been described[2]. The latter cells have the properties of intrathymic 'stem cells' in that they are the first to appear in the embryonic thymus[2,3] and at least some can be shown to give rise, both *in vivo* (ref. 4. and our unpublished data) and *in vitro*[5], to other thymocyte subpopulations. We show here that 50% of Lyt-2$^-$/L3T4$^-$ cells in the adult thymus express receptors for the polypeptide growth hormone interleukin-2 (IL-2)[6] whereas other cells in the thymus do not. Furthermore, immunohistochemical localization studies on frozen sections indicate a disperse distribution of IL-2 receptor-positive cells in both the cortex and medulla. These novel findings have potential implications in the context of current models of differentiation pathways within the thymus.

Very few positive cells (~2%) were observed when unfractionated thymocytes were exposed to anti-IL-2-receptor monoclonal antibody (PC61) and analysed by flow microfluorometry (FMF)(Fig. 1), in line with a previous report[7]. However, when the Lyt-2$^-$/L3T4$^-$ subpopulation was isolated by negative selection with a mixture of monoclonal antibodies and complement, clear staining of 51 ± 3% (mean ±s.d. of three experiments) of the cells was observed (Fig. 1). As Lyt-2$^-$/L3T4$^-$ cells account for 3% of total thymocytes[2], these data suggest (but do not

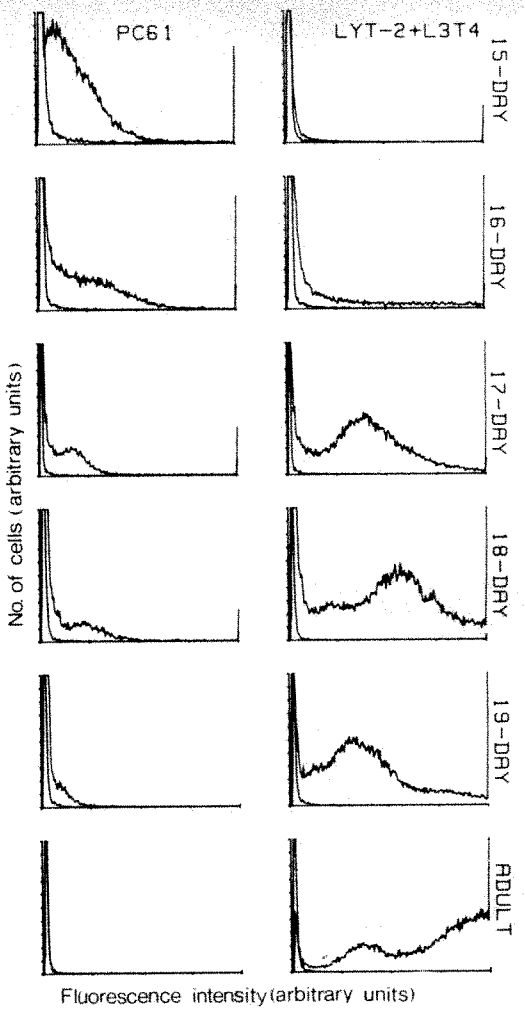


Fig. 2 Ontogeny of IL-2 receptor expression in the developing embryonic thymus. C57BL/6 Thymocytes of the indicated gestational ages, and control adult thymocytes, were stained with PC61 monoclonal antibody (left panels) and analysed by FMF as described in Fig. 1 legend. For comparison, the proportion of Lyt-2$^-$/L3T4$^-$ cells in the same population was determined by simultaneous staining with anti-Lyt-2 and anti-L3T4 monoclonal antibodies[2] (right panels). The proportions of PC61$^+$ and Lyt-2$^-$/L3T4$^-$ cells, respectively, were: day 15 (66%, 88%), day 16 (40%, 60%), day 17 (15%, 30%), day 18 (5%, 15%), day 19 (4%, 10%), adult (2%, 3%).

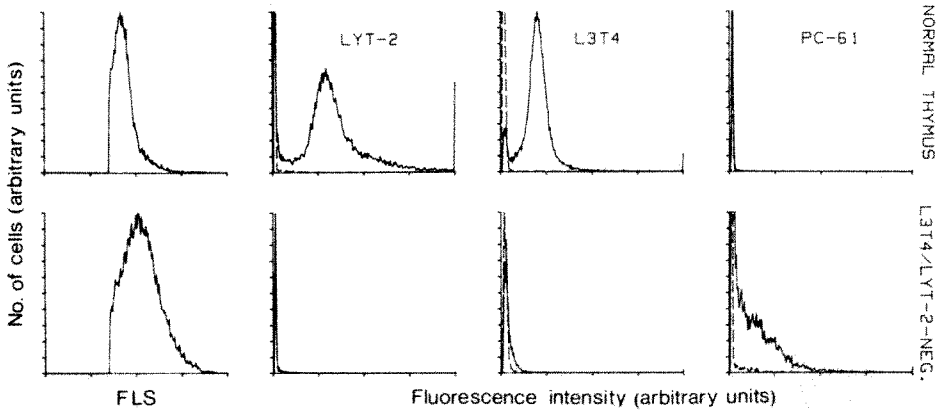


Fig. 1 Flow microfluorimetric (FMF) analysis of IL-2 receptor expression by Lyt-2$^-$/L3T4$^-$ thymocytes (lower row). Upper row, normal thymus controls. Linear fluorescence or forward light scatter (FLS) histograms are expressed in arbitrary units. Dotted lines represent staining with the fluorescent conjugate only.
Methods. C57BL/6 thymocytes (50×10^6 ml^{-1}) were incubated with a mixture of rat IgM monoclonal antibodies directed against Lyt-2 (3.168.8.1[21], provided by F.W. Fitch) and L3T4 (LICR.LAU.RL172.4) at a 1:10 final dilution of hybridoma culture supernatant. The latter monoclonal antibody was obtained by fusing spleen cells from a rat immunized with the L3T4-positive thymoma EL-4 to the non-secreting mouse myeloma X63-Ag8.653 and screening the hybrids for selective complement-dependent lysis of L3T4-positive thymomas (R.C., R. K. Lees and H. R. MacD., unpublished data). After incubation for 15 min at 37 °C, rabbit complement was added to a 1:20 final dilution for an additional 45 min. Treated cells were passed over Ficoll-Hypaque (specific gravity 1.077) and washed twice. Viability was >90%, and cell recovery was 1.0 ± 0.3% (mean ± s.d. of six experiments). For FMF analysis, aliquots of 1×10^6 control or Lyt-2$^-$/L3T4$^-$ thymocytes in 100 μl were incubated at 4 °C with appropriate (saturating) concentrations of rat monoclonal antibodies directed against either Lyt-2 (53-6.7[22], provided by J. Ledbetter), L3T4 (H129-19[23], provided by M. Pierres) or the murine IL-2 receptor (PC61). After 30 min, 50 μg of fluorescein isothiocyanate-conjugated rabbit anti-rat immunoglobulin (Nordic Laboratories) was added for an additional 30 min at 4 °C. Control samples were stained with the second step reagent alone. All samples were then passed on a flow cytometer (FACS II, Becton and Dickinson) gated to exclude nonviable cells[24].

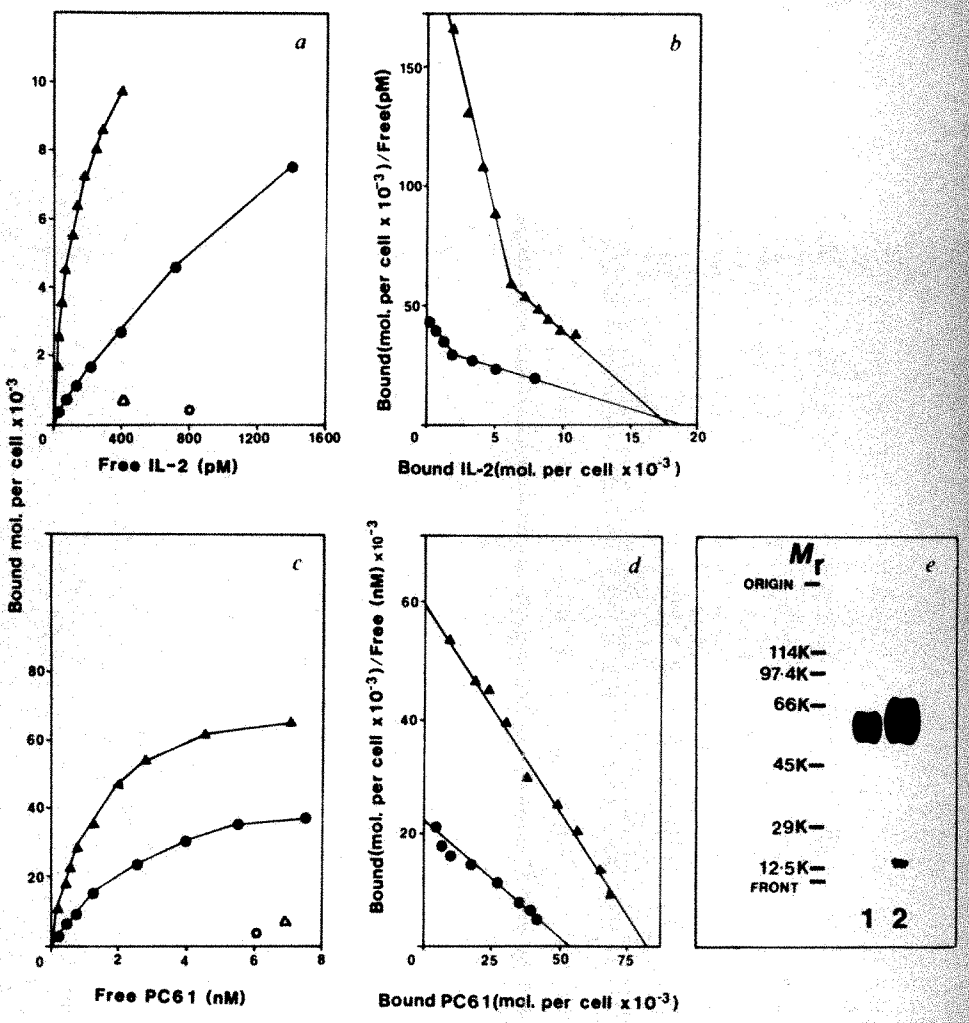


Fig. 3 Equilibrium binding and immunoprecipitation analysis of IL-2 receptors on Lyt-2$^-$/L3T4$^-$ thymocytes and Con A blasts. Lyt-2$^-$/L3T4$^-$ thymocytes (prepared as described in Fig. 1) and Con A blasts (prepared by culturing 2×10^6 C57BL/6 spleen cells for 4 days together with 1.5 μg ml^{-1} Con A and 10% (v/v) supernatant from phorbol myristate acetate-stimulated EL-4 cells) were passed over Ficoll-Hypaque and washed extensively. ^{3}H-IL-2 from biosynthetically labelled Jurkat cell supernatant was purified by immunoaffinity chromatography on a column of Aphigel coupled to DMS-3 anti-IL-2 monoclonal antibody (provided by K. A. Smith) as described in detail elsewhere[25]. ^{3}H-PC61 was purified from biosynthetically labelled hybridoma culture supernatant by dialysis followed by passage over protein A-Sepharose. For the binding assays (*a*–*d*) aliquots of 1–3 $\times10^6$ cells in 200 μl were incubated for 20 min at 4 °C with varying concentrations of ^{3}H-IL-2 or ^{3}H-PC61. Free and cell bound radioactivity was measured by liquid scintillation counting following centrifugation of samples through a paraffin/silicon oil gradient[10]. The number of bound IL-2 and PC61 molecules per cell was calculated given the specific activities of 2,500 c.p.m. ng^{-1} and 210 c.p.m. ng^{-1}, respectively. The M_r of IL-2 and PC61, as judged by SDS-PAGE, is 15,000 (15K) and 170K, respectively. Nonspecific binding was measured by incubating the cells in the presence of a 100-fold excess of the relevant unlabelled ligand. Equilibrium binding of IL-2 (▲, Con A blasts; ●, thymocytes) is shown in *a* after subtraction of non-saturable binding (open symbols). The open symbols represent ^{3}H-IL2 binding in the presence of either excess cold IL-2 or PC61 mAb. For Con A blasts, binding was saturable at a free IL-2 concentration of ~2 nM, whereas the binding to Lyt-2$^-$/L3T4$^-$ thymocytes was not saturable at this concentration. Equilibrium binding of ^{3}H-PC61 is shown in *c* (same symbols as *a*). Scatchard plots of these data are shown in *b* and *d*. The number of high- and low-affinity IL-2 binding sites was estimated by computer analysis of the two linear components of the Scatchard plots. For immunoprecipitation analysis (*e*), Lyt-2$^-$/L3T4$^-$ thymocytes (3×10^6 cells, lane 1) or Con A blasts (10×10^6 cells, lane 2) were labelled by the enzyme catalysed surface iodination technique of Hubbard and Cohn[26] modified as described previously[27]. The labelled cells were lysed in 0.5 ml of cold 1% recondensed Triton X-114 in 50 nM Tris-HCl (*p*H 7.7) containing 0.3 M NaCl and 5 mM EDTA. The aqueous and detergent phases were separated according to Bordier[28]. The lysates were pre-cleared for immunoprecipitation by sequential addition of 5 μl normal rat serum, 20 μl rabbit anti-rat immunoglobulin and 12.5 μl Protein-A-Sepharose 4B (twice). The pre-cleared lysates were then incubated for 60 min with purified PC61 antibody coupled to Sepharose 4B, followed by extensive washing in phosphate buffer (*p*H 8.2). The immunoprecipitates were analysed by SDS-PAGE according to Laemmli[29] on 7.5–15% gradient gels. Samples were reduced with 0.1 M dithiothreitol and denatured by boiling in 4% SDS. After electrophoresis, gels were autoradiographed at −70 °C on Kodak XAR film. Exposure times were 8 days (lane 1) and 4 days (lane 2). M_r standards were β-galactosidase (114K), phosphorylase *b* (97.4K), bovine serum albumin (66K), ovalbumin (45K), carbonic anhydrase (29K) and cytochrome *c* (12.5K).

prove) that all PC61$^+$ cells are contained within this subpopulation. No Lyt-2$^+$ or L3T4$^+$ cells were detected after the antibody treatment; however, ~90% of the cells expressed Thy-1. The fluorescence distribution of PC61$^+$ cells was heterogeneous, with a mean intensity corresponding to 76% of that obtained for IL-2-dependent cells from a cytotoxic T-cell line[8] (included in each experiment as a positive control). Forward light scatter (FLS) analysis of Lyt-2$^-$/L3T4$^-$ cells indicated that they were considerably larger than most thymocytes (124 ± 4 arbitrary units as compared with a value of 100 units for small cortical thymocytes). Simultaneous FLS and PC61 fluorescence analysis of the Lyt-2$^-$/L3T4$^-$ subpopulation did not reveal any size distinction between PC61$^+$ and PC61$^-$ cells.

The early (day 15) embryonic thymus, which is known from transplantation experiments to be a rich source of intrathymic stem cells[9], is also enriched for Lyt-2$^-$/L3T4$^-$ cells[2], but their proportion decreases rapidly during subsequent development[2]. When thymocytes from embryos of differing gestational ages were stained with PC61 and analysed by FMF, a good correlation was observed between the proportion of PC61$^+$ and Lyt-2$^-$/L3T4$^-$ cells (Fig. 2).

Several other independent experimental approaches were used to verify IL-2 receptor expression on Lyt-2$^-$/L3T4$^-$ thymocytes. Equilibrium binding assays using biosynthetically labelled immunoaffinity-purified IL-2 (^{3}H-IL-2) demonstrated that adult Lyt-2$^-$/L3T4$^-$ thymocytes bound IL-2, and that this binding could be competed for by excess unlabelled IL-2 or by purified PC61 antibody (Fig. 3*a*). This latter result further confirms the fact that PC61 reacts with the IL-2 receptor. Scatchard plot analysis of the IL-2 binding data (Fig. 3*b*) shows that Lyt-2$^-$/L3T4$^-$ thymocytes express two classes of receptors with differing affinity for IL-2 (apparent dissociation constant, K_D, of 96 and 8,130 pM). The total number of IL-2 binding sites per cell was about 14,700, with 1,200 receptors being of the high-

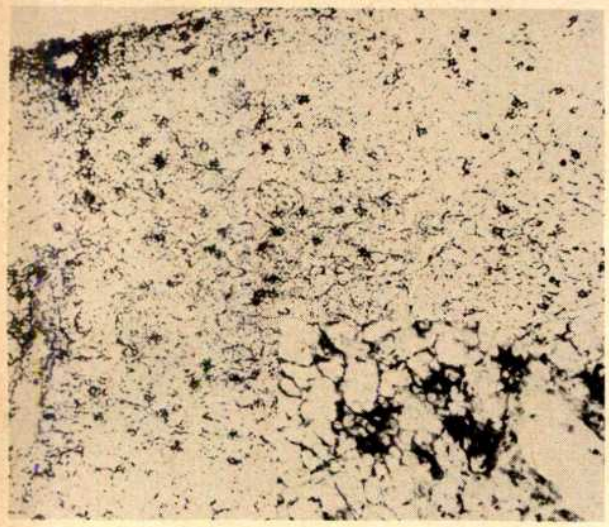


Fig. 4 *In situ* localization of PC61$^+$ cells in the thymus. Thymuses from 6-week-old C57BL/6 mice were removed and snap frozen as previously described[17]. PC61 monoclonal antibody was directly coupled to biotin and the bound antibody revealed by adding an avidin–peroxidase conjugate[30] followed by freshly prepared 3,3 diaminobenzidine solution containing 0.06% H_2O_2. At low magnification (×450), positively stained cells are seen as individual cells and small clusters scattered throughout the cortex. Insert (×890): positive cells are also seen surrounding small blood vessel (bottom right) in medulla.

affinity class. By comparison, concanavalin A (Con A)-activated T lymphocytes expressed slightly more IL-2 receptors (37,000 total and 3,000 high-affinity binding sites) and bound ^{3}H-IL-2 with higher affinity (K_D = 19 and 1,360 pM for high- and low-affinity receptors, respectively). Previous reports indicated that activated T lymphocytes expressed only a single class of high-affinity IL-2 receptors[10]; however, it has recently become clear that activated T lymphocytes express two classes of receptors when very high concentrations of ^{3}H-IL-2 are used[11].

The significance of the reduced affinity of IL-2 receptors on adult Lyt-2$^-$/L3T4$^-$ thymocytes compared with Con A blasts is not known. For antigen- or mitogen-activated mature T cells and IL-2-dependent T-cell lines, there is a good quantitative correlation between the K_D of high-affinity IL-2 receptors (~20 pM) and the IL-2 concentration required for half-maximal proliferation[6] (by definition, 1 unit ml^{-1}). In the present case, saturation of IL-2 receptors on Lyt-2$^-$/L3T4$^-$ cells would be more difficult to achieve because of their low affinity (K_D = 96 pM), and indeed we and others (see accompanying paper[12]) have thus far observed only marginal proliferation (as judged by ^{3}H-thymidine incorporation) when such cells are cultured in the presence of extremely high concentrations (100–300 units ml^{-1}) of affinity-purified or recombinant IL-2.

Equilibrium binding with biosynthetically labelled purified PC61 monoclonal antibody (^{3}H-PC61) confirmed and extended the fluorescence results. Figure 3*c* shows that ^{3}H-PC61 binding to adult Lyt-2$^-$/L3T4$^-$ thymocytes and Con A blasts is saturable and can be competed for by excess unlabelled PC61 antibody. From Scatchard plots of these data (Fig. 3*d*), the total number of PC61 binding sites is 53,000 for thymocytes and 81,000 for Con A blasts, and both cell populations bind the monoclonal antibody with similar affinity (K_D of 2.1 nM and 1.3 nM, respectively). The reason for the apparent excess of PC61 over IL-2 binding sites is not clear, but a similar discrepancy has been observed for IL-2[10] and anti-Tac[13] binding to human HUT-102 cells.

Analysis by SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions (Fig. 3*e*) illustrates that PC61 monoclonal antibody precipitates membrane components of similar apparent relative molecular mass (M_r = 55,000–65,000) from adult Lyt-2$^-$/L3T4$^-$ thymocytes and Con A blasts. These results are similar to those reported for a variety of human and mouse T-cell lines[7,13]. In addition, bands of lower M_r (15,000–25,000) were precipitated from Con A blasts. The nature of this low-M_r material is unknown, but we find similar results with other T-cell lines and with an independently derived anti-IL-2-receptor monoclonal antibody (7D4[7], kindly provided by T. R. Malek).

The apparently unique reactivity of PC61 antibody with Lyt-2$^-$/L3T4$^-$ thymocytes provides an opportunity to determine the anatomical location of these putative stem cells *in situ*. Immunohistochemical localization of PC61$^+$ cells by the peroxidase method (Fig. 4) shows that individual cells (or small clusters) can be seen scattered more or less at random throughout both the thymic cortex and medulla. This distribution is at odds with the widely held view that thymic maturation proceeds sequentially from the subcapsular region through the cortex to the medulla[14]; however, it is consistent with early studies by Metcalf[15] as well as the recent data of Ezine *et al.*[16] and Ceredig and Schreyer[17] suggesting a focal (clonal?) distribution of donor-derived (putative stem) cells repopulating the thymus in Thy-1 congenic radiation bone marrow chimaeras. It remains to be determined whether the foci of IL-2- receptor-bearing cells observed here and as recently reported by Takacs *et al.*[31] are associated with a particular non-lymphoid component of the thymus.

The presence of IL-2 receptors on Lyt-2$^-$/L3T4$^-$ thymocytes raises interesting questions relevant to T-cell differentiation and activation. Among mature T cells, IL-2 receptor expression is always linked to an activation process dependent on antigen[18] or mitogen[19] binding. In this context, the apparently 'spontaneous' expression of IL-2 receptors on a subpopulation of intrathymic stem cells raises the possibility that receptor expression during the early stages of T-cell differentiation is not yet linked to specific antigenic stimulation. Alternatively, IL-2 receptor expression by these cells may reflect some transient 'antigen-like' differentiative stimulus (either cellular or humoral) intrinsic to the thymus. If the latter hypothesis is correct, the data presented here provide evidence that the T-cell specificity repertoire may result from *in situ* selection (either positive or negative) by self antigens within the thymus[20].

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1. Cantor, H. & Weissman, I. L. *Prog. Allergy* **20,** 1–64 (1976).
2. Ceredig, R. *et al. J. exp. Med.* **158,** 1654–1671 (1983).
3. Mathieson, B. J. *et al. Nature* **289,** 179–181 (1981).
4. Mathieson, B. J. & Fowlkes, B. J. *Immun. Rev.* **82,** (1984).
5. Ceredig, R., Sekaly, R. P. & MacDonald, H. R. *Nature* **303,** 248–250 (1983).
6. Smith, K. A. *A. Rev. Immun.* **2,** 319–333 (1984).
7. Malek, T. R., Robb, R. J. & Shevach, E. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5694–5698 (1983).
8. Gillis, S. *et al. J. Immun.* **120,** 2027–2032 (1978).
9. Owen, J. J. T. & Jenkinson, E. J. *Prog. Allergy* **29,** 1–27 (1981).
10. Robb, R. J., Munck, A. & Smith, K. A. *J. exp. Med.* **154,** 1455–1474 (1981).
11. Robb, R. J., Greene, W. C. & Rusk, C. M. *J. exp. Med.* **160,** 1126–1146 (1984).
12. Raulet, D. H. *Nature* **314,** 101–103 (1985).
13. Leonard, W. J. *et al. Nature* **300,** 267–269 (1982).
14. Weissman, I. L. *J. exp. Med.* **137,** 504–510 (1973).
15. Metcalf, D. in *The Thymus: Its Role in Immune Responses, Leukaemia Development and Carcinogenesis,* 1–17 (Springer, Heidelberg, 1966).
16. Ezine, S., Weissman, I. L. & Rouse, R. V. *Nature* **309,** 629–631 (1984).
17. Ceredig, R. & Schreyer, M. *Thymus* **6,** 15–26 (1984).
18. Hemler, M. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 2172–2175 (1984).
19. Cantrell, D. & Smith, K. A. *J. exp. Med.* **158,** 1895–1911 (1983).
20. Jerne, N. K. *Eur. J. Immun.* **1,** 1–9 (1971).
21. Sarmiento, M., Glasebrook, A. L. & Fitch, F. W. *J. Immun.* **125,** 2665–2672 (1980).
22. Ledbetter, J. A. & Herzenberg, L. A. *Immun. Rev.* **47,** 63–90 (1979).
23. Pierres, A. *et al. J. Immun.* **132,** 2775–2782 (1984).
24. MacDonald, H. R. & Zaech, P. *Cytometry* **3,** 55–58 (1982).
25. Smith, K. A., Favata, M. F. & Oroszlan, S. *J. Immun.* **131,** 1808–1815 (1983).
26. Hubbard, A. L. & Cohn, Z. A. *J. cell Biol.* **64,** 438–460 (1975).
27. Luescher, B. *et al. Molec. Immun.* **21,** 329–336 (1984).
28. Bordier, C. *J. biol. Chem.* **256,** 1604–1607 (1981).
29. Laemmli, U. K. *Nature* **221,** 680–685 (1970).
30. Heitzmann, H. & Richards, R. M. *Proc. natn. Acad. Sci.* **71,** 3537–3541 (1974).
31. Takacs, L., Osawa, H., Törö, I. & Diamenstein, T. *Clin. Exp. Immunol.* **59,** 37–44 (1985).

Expression and function of interleukin-2 receptors on immature thymocytes

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T-cell differentiation represents a unique system for studying mechanisms of lymphoid development because it occurs in a segregated site, the thymus, in which distinct subpopulations of thymocytes at various stages of differentiation can be defined on the basis of the differential expression of T-cell surface antigens as well as topography. There is particular interest in thymocyte differentiation because the genotype of radioresistant thymus cells influences the specificity repertoire of the pool of T cells that mature therein: that is, the major histocompatibility complex (MHC) antigens expressed by thymus cells bias the pool of maturing T cells towards recognition of antigens in the 'context' of the products of that MHC haplotype ('thymus education'; refs 1–3). Immature T cells with affinity for thymus MHC antigens are generally thought to undergo a stage of positive selection in the thymus[4–6]. Here we report that 30% of cells in the least mature adult thymocyte subpopulation yet defined, as well as 50% of immature fetal thymocytes, express receptors for interleukin-2 (IL-2, the T-cell growth factor) without *in vitro* induction, and will proliferate vigorously in an IL-2-dependent fashion if provided with co-stimulating mitogen.

Recent studies with monoclonal antibodies reactive with the T-cell antigens Lyt-2, L3T4 and Thy-1 have defined at least four subpopulations of thymocytes in the adult thymus. Thymocytes with the mature phenotypes L3T4$^+$, Lyt-2$^-$, Thy-1$^+$ (helper T-cell phenotype) or L3T4$^-$, Lyt-2$^+$, Thy-1$^+$ (cytolytic T-cell phenotype) appear late in fetal ontogeny[7,8] and most are functional in *in vitro* assays of T-cell activities[7,9,10]. These cells may include end-stage thymocytes awaiting export from the thymus although medullary thymocytes with these phenotypes may not be exported[11]. A large subpopulation (~85%) of thymocytes are mostly small noncycling cells with the phenotype L3T4$^+$, Lyt-2$^+$, Thy-1$^+$, a phenotype not yet found amongst peripheral T cells; the specific ontogenetic relationship of these cells to other thymocyte subpopulations (that is, whether they are intermediates) is presently obscure. A fourth thymus subpopulation (~3% of adult thymocytes) has the phenotype L3T4$^-$, Lyt-2$^-$, Thy-1$^+$ and includes mostly blast cells, many of which are cycling[12]. In addition to the immature phenotype of these cells, there is evidence that they represent the least mature thymocyte subpopulation yet defined: on transfer to irradiated recipients these cells, unlike other thymocytes, are able to repopulate the recipient thymus and give rise with time to each of the other thymocyte subpopulations[12]. Furthermore, before day 17 of embryonic murine development most (80–95%) thymocytes have the L3T4$^-$, Lyt-2$^-$, Thy-1$^+$ phenotype[7,8].

In our studies of the expression of IL-2 receptors by thymocytes, we used the recently described rat monoclonal antibodies 3C7 (IgG) and 7D4 (IgM) isolated by Malek and Shevach[13,14]. These antibodies recognize different epitopes on the IL-2 receptor as they do not compete with each other for binding, and the binding of 3C7 (but not 7D4) is blocked by purified IL-2: both antibodies block IL-2-dependent proliferation and precipitate a similar polypeptide of relative molecular mass 50,000–60,000 (refs 13, 14).

Indirect immunofluorescent staining profiles of day 16 fetal thymocytes and adult lymph node cells with various monoclonal antibodies (Fig. 1) show that most fetal thymocytes (95%) are Lyt-2$^-$, Thy-1$^+$, whereas adult thymocytes are predominantly Lyt-2$^+$, Thy-1$^+$ cells. About 50% of fetal thymocytes bind the 3C7 anti-IL-2 receptor antibody and 30% bind the 7D4 antibody.

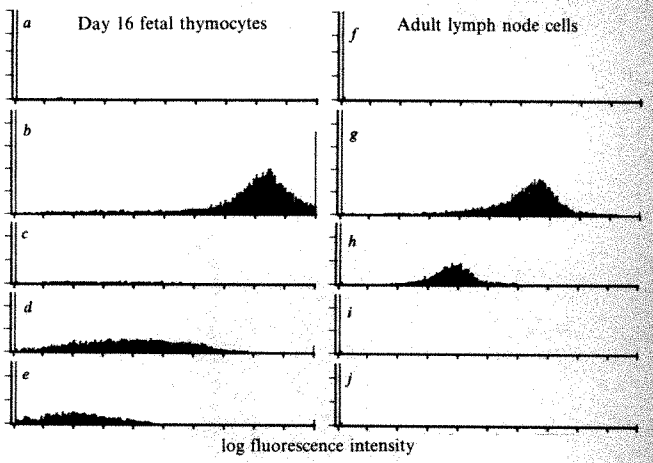


Fig. 1 Fetal thymocytes express IL-2 receptors. BALB/cAnN fetal thymocytes (*a–e*) from day 16 of gestation (day 0 is the day of observation of vaginal plugs) or adult (8 weeks old) lymph node cells (*f–j*) were stained indirectly with the following rat monoclonal antibodies: *a, f*, no antibody; *b, g*, anti-Thy-1 (T24, a rat/mouse hybridoma secreting IgG anti-Thy-1, provided by Dr Ian Trowbridge); *c, h*, anti-Lyt-2 (53-6.7)[17]; *d, i*, anti-IL-2 receptor (3C7)[14]; *e, j*, anti-IL-2 receptor (7D4)[13,14].
Methods. Cell suspensions (1×10^6 cells in 50 μl) were incubated in phosphate-buffered saline (PBS) containing 5% fetal calf serum (FCS), 0.1% NaN_3 for 30 min at 4 °C with or without monoclonal antibodies, washed three times, and resuspended in 50 μl of the same medium containing 25 μg ml^{-1} affinity-purified, mouse serum absorbed fluorescent goat anti-mouse IgG antibodies (Kirkegaard and Perry, Gaithersburg, Maryland). Following incubation for 30 min at 4 °C, the cells were washed three times, resuspended in 0.5 ml of the PBS–FCS–NaN_3 solution and analysed by flow microfluorimetry with an Ortho Cytofluorograf System 50 interfaced with an Ortho 2150 computer. The antibodies were used at the following excess concentrations as determined in pilot experiments: T24, 1/10 dilution of culture supernatant; 53-6.7, 1/5 dilution of 10-fold concentrated culture supernatant; 3C7 and 7D4, 1/50 dilution of ascites fluid.

(The smaller proportion of cells positive with 7D4 antibodies and the weaker staining intensity of these cells probably results from the inferior binding of our secondary fluorescent goat-anti-rat IgG reagent to IgM molecules; data not shown.) In contrast, expression of IL-2 receptors by adult lymph node cells is nearly undetectable (as in unstimulated splenic T cells[14]), reflecting the need for antigenic or mitogenic stimulation for expression of IL-2 receptors by mature T cells.

To assess expression of IL-2 receptors by immature thymocytes in the adult thymus, we purified L3T4$^-$, Lyt-2$^-$ thymocytes by two rounds of lysis of whole thymocyte populations with monoclonal anti-L3T4 (GK 1.5[15]) and anti-Lyt-2 (AD4(15)[16]) antibodies plus complement and stained them indirectly with monoclonal antibodies. Flow cytometric analysis of these cells, with unfractionated thymocytes for comparison (Fig. 2), indicates that cells prepared in this way are routinely ~95% Lyt-2$^-$, L3T4$^-$, Thy-1$^+$ cells. By forward-angle scatter measurements, L3T4$^-$, Lyt-2$^-$ thymocytes are larger than most unfractionated thymocytes (refs 7, 12 and our unpublished data) and express Thy-1 at high levels. The background staining of these cells in the negative control results from a small number of cells (about 3–5%) binding small amounts of anti-L3T4 or anti-Lyt-2 and which presumably escaped complement lysis. As can be seen, about 30% of L3T4$^-$, Lyt-2$^-$ thymocytes stain with the 3C7 and 7D4 antibodies reactive with the IL-2 receptor. In contrast, very few unfractionated thymocytes stain with these reagents[14] (Fig. 1). However, a few positive cells, with forward-angle scatter characteristics consistent with the size of L3T4$^-$, Lyt-2$^-$ thymocytes, can be seen in plots of forward-angle scatter versus fluorescence (not shown). In three experiments the proportion of positive cells in unfractionated thymocyte populations

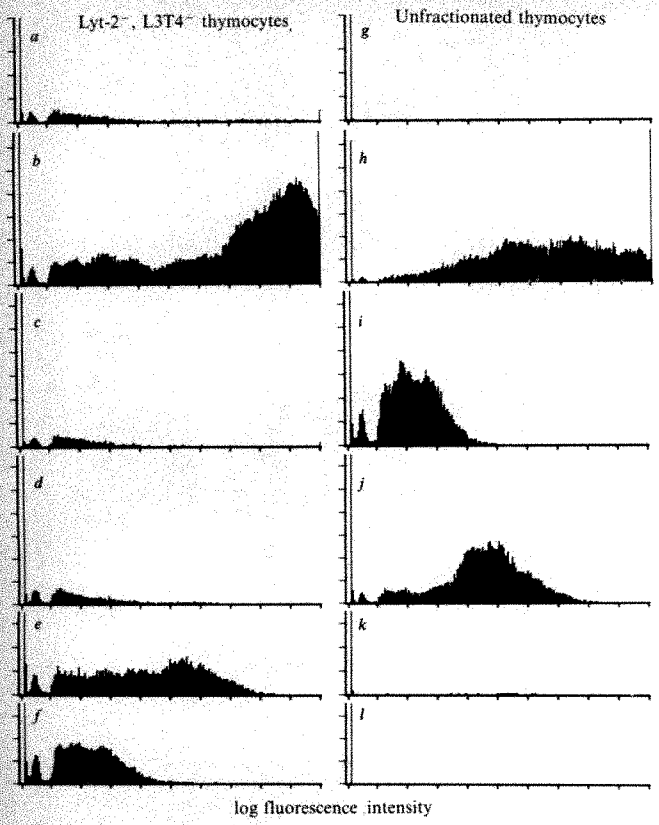


Fig. 2 Immature thymocytes in the young adult thymus express IL-2 receptors. Lyt-2$^-$, L3T4$^-$ thymocytes (*a–f*) or unfractionated thymocytes (*g–l*) from 4-week-old BALB/cAnN mice were stained indirectly as described in Fig. 1 legend with the following rat monoclonal antibodies: *a*, *g*, no antibody; *b*, *h*, anti-Thy-1 (T24); *c*, *i*, anti-L3T4 (1/20 dilution of 10-fold concentrated culture supernatant from the GK 1.5 hybridoma); *d*, *j*, anti-Lyt-2 (53-6.7); *e*, *k*, anti-IL-2 receptor (3C7); *f*, *l*, anti-IL-2 receptor (7D4). Concentrations of antibodies as in Fig. 1.

Methods. Lyt-2$^-$, L3T4$^-$ thymocytes were isolated from thymocyte suspensions by two consecutive treatments with ascites fluids (1/100 dilution) of anti-Lyt-2.2 (AD4(15))[16] and anti-L3T4 (GK 1.5) plus complement (C). Cells at a concentration of 1×10^7 ml^{-1} in RPMI-1640 medium, 5% FCS, were incubated with antibodies at 4 °C for 30 min, washed once and resuspended in a mixture of guinea pig complement (Gibco) at a 1/10 dilution and selected rabbit complement at a 1/50 dilution. Following incubation for 45 min at 37 °C, the surviving cells (about 2% of the starting number) were purified on step gradients of Ficoll–Isopaque[18], washed, and the entire procedure was repeated except that anti-I-A^d (1/100 dilution of ascites fluid of BP107)[19] was included in the antibody cocktail in order to deplete any B cells or macrophages in the cell preparation. The overall yield of cells from the treatments was ~1% in several experiments.

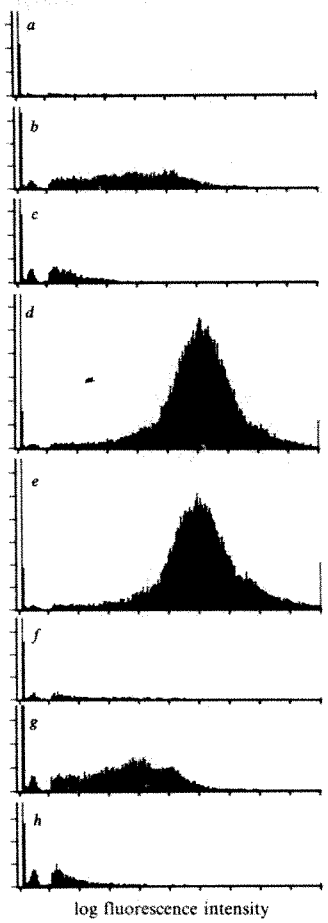


Fig. 3 IL-2 blocks binding of anti-IL-2 receptor antibodies to immature thymocytes. 1×10^6 day 16 fetal thymocytes (*a–e*) or young adult Lyt-2$^-$, L3T4$^-$ thymocytes (*f–h*) isolated as described in the legends to Figs 1 and 2, were preincubated for 30 min at room temperature with (*c*, *e*, *h*) or without (*a*, *b*, *d*, *f*, *g*) 1 μg purified, bacterially derived recombinant human IL-2 (AmGen Biologicals) in 50 μl of PBS–FCS–NaN_3. Rat monoclonal antibodies were then added directly to the wells for indirect immunofluorescent staining as described in Fig. 1 legend. *a*, *f*, No antibody; *b*, *c*, *g*, *h*, a limiting (1/200) dilution of ascites fluid of the 3C7 anti-IL-2 receptor monoclonal antibody; *d*, *e*, a limiting (1/50) dilution of culture supernatant of anti-Thy-1 (T24) hybridoma.

averages ~1.0%. As L3T4$^-$, Lyt-2$^-$ thymocytes represent 3% of adult thymocytes and ~30% of these cells stain with anti-IL-2 receptor monoclonal antibody, it appears that a large fraction (or perhaps all) of IL-2 receptor-positive thymocytes are in the L3T4$^-$, Lyt-2$^-$ subpopulation.

As immature thymocytes are bound by two different monoclonal antibodies reactive with different epitopes on the IL-2 receptor, we predicted that these cells expressed IL-2 receptors or closely related structures. To establish whether the putative receptors bind IL-2, we exploited the observation that binding of the 3C7 monoclonal antibody to the IL-2 receptor can be blocked with purified IL-2[14]. Day 16 fetal thymocytes were stained indirectly with a limiting amount (as determined in a previous experiment) of 3C7 or anti-Thy-1 monoclonal antibodies in the presence or absence of 1 μg purified recombinant human IL-2. As shown in Fig. 3*a–e*, the binding of 3C7 monoclonal antibody but not anti-Thy-1 to fetal thymocytes is profoundly inhibited by the purified IL-2. Virtually identical results were obtained in a similar analysis of L3T4$^-$, Lyt-2$^-$ thymocytes isolated from young adult thymuses (Fig. 3*f–h*). The data, taken together, show that immature thymocytes express IL-2-binding receptors that express epitopes similar to their counterparts on activated mature peripheral T cells.

We next determined the density of IL-2 receptors on these immature thymocytes relative to concanavalin A (Con A)-activated peripheral T cells. We used the 7D4 monoclonal antibody as its binding is not inhibited by IL-2[14]. The antibody was used at a previously determined excess concentration. The relative level of staining of L3T4$^-$, Lyt-2$^-$ young adult thymocytes (a mean of 41 arbitrary fluorescence units on a linear scale) corresponded closely to the level of staining of Con A blasts (50 fluorescence units). Because in several experiments the level of IL-2 receptors on day 16 fetal thymocytes was virtually indistinguishable from the levels on L3T4$^-$, Lyt-2$^-$ young adult thymocytes, the fetal thymocytes also seem to express levels of receptor comparable with Con A blasts. These results indicate that IL-2 receptor expression by these immature thymocytes is at a level consistent with physiological function.

In further experiments we sought to demonstrate IL-2-dependent proliferation of adult or fetal Lyt-2$^-$, L3T4$^-$ thymocytes (Table 1). Given that immature thymocytes express high con-

centrations of IL-2 receptors, we were surprised to find that the cells responded only weakly to purified recombinant IL-2. Interestingly, a vigorous proliferative response of immature thymocytes to IL-2 was revealed by co-stimulation with the T-cell mitogen Con A, whereas the cells responded only weakly to Con A alone; this was true of both adult and fetal L3T4$^-$, Lyt-2$^-$ thymocytes. However, in two experiments the response of the fetal thymocytes to IL-2 titrated more steeply than that of the young adult L3T4$^-$, Lyt-2$^-$ thymocytes. Whether this reflects differences in receptor affinity or some other difference in these cell populations is unknown. We note that inclusion of irradiated T-cell-depleted syngeneic splenic accessory cells did not augment the response to Con A plus IL-2, nor did the accessory cells substitute for either Con A or IL-2 in stimulating the proliferative response (data not shown).

The expression of apparently functional IL-2 receptors by immature thymocytes suggests that IL-2 plays a role in thymus cell ontogeny. Of obvious interest in this context is the ontogeny of IL-2-producing cells in the thymus. IL-2-producing thymocytes are not detected before day 19 of fetal ontogeny and seem to correlate with the L3T4$^+$, Lyt-2$^-$ subpopulation[7], but it is not clear whether the methods of that study used appropriate conditions to detect IL-2 production by immature thymocytes. We are now assessing other protocols for stimulating IL-2 production by immature thymocytes.

A possible role of IL-2 in the positive selection of thymocytes is suggested by the requirement of Con A for potent proliferative responses to IL-2 despite the fact that the cells express IL-2 receptors without *in vitro* induction. In the case of mature resting T cells, which do not constitutively express IL-2 receptors, mitogenic or antigenic stimulation leads to expression of IL-2 receptors and subsequent IL-2-dependent proliferation. Although expression of IL-2 receptors above a threshold level may be a sufficient condition for IL-2-dependent proliferation of mature T cells, our data suggest that additional requirements, provided by mitogenic stimulation, are operative, at least in the case of immature thymocytes. Con A may act by stimulating cell surface structures on immature thymocytes involved in recognition of MHC antigens and/or nominal antigens. *In vivo*, stimulation of the receptor might aid positive selection of thymocytes during the 'education' of thymocytes.

Finally, the present results do not imply that all thymocyte proliferation is IL-2 dependent, as thymic blasts with phenotypes distinct from the L3T4$^-$, Lyt-2$^-$ subpopulation have been reported[7,11].

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1. Zinkernagel, R. M. *Immun. Rev.* **42**, 224-270 (1978).
2. Bevan, M. J. & Fink, P. J. *Immun. Rev.* **42**, 3-19 (1978).
3. Sprent, J. *Immun. Rev.* **42**, 108-137 (1978).
4. Jerne, N. K. *Eur. J. Immun.* **1**, 1-9 (1973).
5. Bevan, M. J. *Immun. Today* **2**, 216-219 (1981).
6. Janeway, C. A., Wigzell, H. & Binz, H. *Scand. J. Immun.* **5**, 993-1001 (1976).
7. Ceredig, R., Dialynas, D., Fitch, F. & MacDonald, H. R. *J. exp. Med.* **158**, 1654-1671 (1983).
8. Mathieson, B. J., Sharrow, S. O., Rosenberg, V. & Hammerling, U. *Nature* **289**, 179-181 (1981).
9. Ceredig, Rh., Glasebrook, A. L. & McDonald, H. R. *J. exp. Med.* **155**, 358 (1982).
10. Shortman, K., Scollay, R., Chen, W.-F. & Andrews, P. *J. cell. Biochem.* Suppl. 8A, 110 (1984).
11. Reichert, R. A., Gellatin, W. M., Butcher, E. C. & Weissman, I. L. *Cell* **38**, 89-99 (1984).
12. Fowlkes, B. J., Edison, L., Chesed, T. & Mathieson, B. J. *J. cell. Biochem.* Suppl. 8A, 108 (1984).
13. Malek, T. R., Robb, R. J. & Shevach, E. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5694-5698 (1983).
14. Ortega, R. G., Robb, R. J., Shevach, E. M. & Malek, T. R. *J. Immun.* **133**, 1970-1975 (1984).
15. Dialynas, D. P. *et al. Immun. Rev.* **74**, 29-56 (1983).
16. Raulet, D. H., Gottlieb, P. D. & Bevan, M. J. *J. Immun.* **125**, 1136-1143 (1980).
17. Ledbetter, J. A. & Herzenberg, L. A. *Immun. Rev.* **47**, 63-85 (1979).
18. Davidson, W. F. & Parish, C. R. *J. immun. Meth.* **7**, 281-289 (1975).
19. Symington, F., Mckearn, T. & Sprent, J. *Immunogenetics* **14**, 53-58 (1981).
20. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. *J. Immun.* **120**, 2027-2032 (1978).

Table 1 Proliferative responses of Lyt-2$^-$, L3T4$^-$ thymocytes

Stimulant in culture	Lyt-2$^-$, L3T4$^-$ thymocytes	
	Adult	Day 16 fetal
None	91 ± 17	52 ± 4
IL-2*, 5 U ml^{-1}	4,518 ± 701	1,220 ± 201
IL-2, 1 U ml^{-1}	2,477 ± 84	294 ± 61
IL-2, 0.2 U ml^{-1}	1,131 ± 96	246 ± 187
Con A†	253 ± 26	924 ± 85.7
Con A + IL-2, 5 U ml^{-1}	28,282 ± 1,128	18,020 ± 1,610
Con A + IL-2, 1 U ml^{-1}	18,855 ± 1,114	1,464 ± 401
Con A + IL-2, 0.2 U ml^{-1}	6,073 ± 634	215 ± 45

Thymocytes were treated once (fetal thymocytes) or twice consecutively (young adult thymocytes) with anti-Lyt-2 and anti-L3T4 monoclonal antibodies plus complement (see Fig. 2) and viable cells purified on gradients of Ficoll-Isopaque. Recoveries from complement lysis were 1.3% and 68% for the young adult and fetal cells respectively. In tests of stimulant activity, triplicate cultures of cells in round-bottom microtitre wells (Costar 3799 plates) contained 1×10^5 cells in 0.2 ml RPMI 1640 medium, 5% fetal calf serum, 50 μM 2-mercaptoethanol, 0.02% glutamine, antibiotics and the additions indicated. After culture for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air, 0.5 μCi ^{3}H-thymidine (6.7 Ci mmol^{-1}) was added to each well and the cultures continued for 4 h, at which time the cells were collected and lysed on glass fibre filters and counted by liquid scintillation spectrometry.

* Purified human recombinant IL-2 (AmGen Biologicals) was titred in our laboratory and compared with a standard sample of IL-2 provided by Dr Richard Robb (Dupont, Nemours and Co.) which is referenced against the unit defined by Gillis and Smith[20]. The specific activity of IL-2 we calculate to be 2×10^5 U per mg protein.

† Con A (Pharmacia) was added to a final concentration of 2 μg ml^{-1}.

Developmental regulation of T-cell receptor gene expression

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In contrast to B cells or their antibody products, T lymphocytes have a dual specificity, for both the eliciting foreign antigen and for polymorphic determinants on cell surface glycoproteins encoded in the major histocompatibility complex (MHC restriction)[1-4]. The recent identification of T-cell receptor glycoproteins[5-7] as well as the genes encoding T-cell receptor subunits will help to elucidate whether MHC proteins and foreign antigens are recognized by two T-cell receptors or by a single receptor. An important feature of MHC restriction is that it appears to be largely acquired by a differentiating T-cell population under the influence of MHC antigens expressed in the thymus[8-10], suggesting that precursor T cells are selected on the basis of their reactivity with MHC determinants expressed in the host thymus[9-11]. To understand this process of 'thymus education', knowledge of the developmental regulation of T-cell receptor gene expression is necessary. Here we report that whereas messenger RNAs encoding the β-and γ-subunits are relatively abundant in immature thymocytes, α mRNA levels are very low. Interestingly, whereas α mRNA levels increase during further development and β mRNA levels stay roughly constant, γ mRNA falls to very low levels in mature T cells, suggesting a role for the γ gene in T-cell differentiation.

cDNA clones encoding the α- and β-subunits of the T-cell receptor have been isolated[12-17] and identified by comparison with partial protein sequences[18-20]. As reported elsewhere, the predicted primary structures of the β- and α-subunits and, in the case of the β-subunit, the genomic organization of the corresponding genetic elements show striking similarities to their

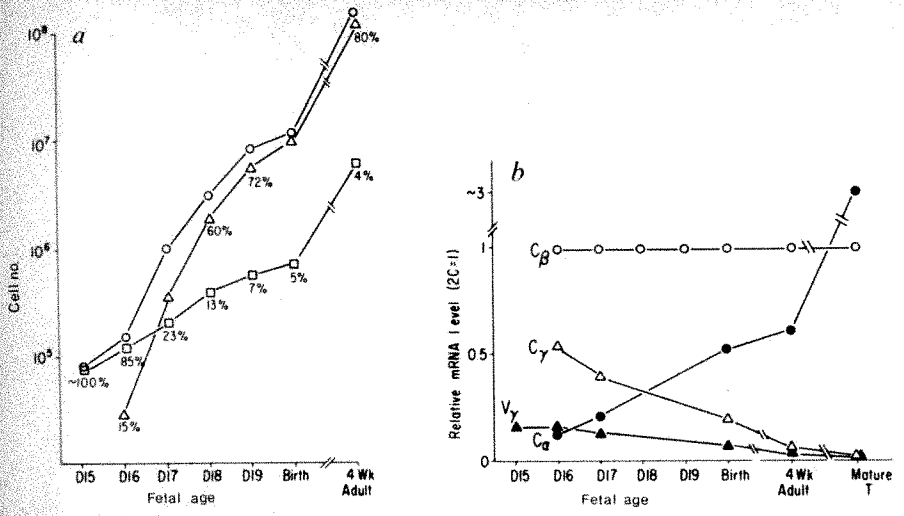


Fig. 1 *a*, Contribution of different subpopulations to thymocyte differentiation. Circles represent the approximate total cellularity of the thymus at different stages; triangles and squares represent the approximate contribution, at each stage, of 'double-positive' and 'double-negative' thymocytes, respectively. The numbers beneath the symbols represent percentages of the total. Data from Ceredig *et al.*[27]. *b*, Approximate relative levels of α, β and γ mRNAs in thymocytes and T cells as a function of fetal and adult age. Data are from the present study. The levels are relative to RNA from the 2C line, and are determined primarily from the Northern data (Figs 3, 4) with reference to the ribonuclease assay data (Fig. 2).

immunoglobulin counterparts[12-17,21-24]. A third gene, γ, has striking similarities to α and β, and may encode an additional T-cell receptor subunit[15,25,26].

Figure 1 represents our depiction of the results of Ceredig *et al.*[27] on the representation of different thymocyte subpopulations during murine development. Thymocytes with the Lyt-2$^-$, L3T4$^-$, Thy-1$^+$ cell surface phenotype (hereafter referred to as 'double-negative' thymocytes) represent the large majority of thymocytes before day 17 of fetal development, and decrease in proportional representation during development until, in the young adult (4-week-old) thymus, they represent only ~4% of the total. After day 16 of fetal development, most thymocytes express the Lyt-2$^+$, L3T4$^+$ ('double-positive') phenotype. Thymocytes with the phenotypes characteristic of mature T cells (Lyt-2$^+$, L3T4$^-$ and Lyt-2$^-$, L3T4$^+$) are detectable shortly after this time and increase in numbers during development to represent ~15% of young adult thymocytes.

The exact lineage interrelations of these subpopulations and the identity of the direct precursor of mature peripheral T cells remain controversial issues, but recent evidence shows that double-negative thymocytes are precursors of the other thymocyte subpopulations[28], and presumably of peripheral T cells. In the analyses reported here, we examine RNA from thymocytes of embryos of days 15, 16, 17 and 20 (newborn) of fetal development, and of 4-week old mice. We also examine RNA from purified double-negative thymocytes from 4-week-old mice and mature lymph-node T-cell RNAs.

T-cell receptor mRNAs were detected by Northern blot analysis and with a recently developed ribonuclease protection assay. We used RNA from the cloned alloreactive cytotoxic T-lymphocyte (CTL) line 2C (ref. 29; see also refs 15, 16) as a standard in each experiment. Titrations of 2C RNA were included so that the relative amounts of each mRNA could be estimated. As a basis for comparison, the frequencies of β and γ cDNA clones in an unselected 2C cDNA library were estimated at 0.1% and 0.02% respectively (H.S., unpublished). The library has not been screened with an α probe, but the frequency of α clones in a subtracted 2C cDNA library is one-half that of β clones[14].

The β probe corresponded to the last third of the β constant region and the 3′-untranslated region of the 3′-most of the two C_β genes ($C_{\beta'}$) (Fig. 2*a*; refs 15, 30, 31); as these are the regions of least homology between the two genes (69%), our assays probably selectively detect transcripts of the $C_{\beta'}$ gene. Analysis of β-chain RNA in developing thymocytes, using the ribonuclease protection assay, showed that β-chain constant-region transcripts are already abundant at day 15 of fetal development (Fig. 2*a*), corresponding to about three times the level found in the reference 2C CTL line. The level of C_β-RNA increases somewhat on day 17 of fetal development and remains approximately constant throughout further thymocyte development. Northern blot analysis showed that approximately two-thirds of the β transcripts in RNA from day 16 fetal thymocytes are 1.0 kilobases (kb) long, and about one-third of the transcripts are 1.3 kb long (Fig. 3*a*). Studies in the mouse[24] and human[32,33] systems suggest that the functional *V-D-J-C* transcripts of β are 1.3 kb long whereas the 1.0-kb mRNA corresponds to transcripts from incompletely rearranged loci. We estimate the level of 1.3-kb β mRNA in day 16 fetal thymocytes to be about equal to the level in RNA from the 2C cell line. The level of 1.3-kb β mRNA is roughly constant throughout further thymocyte development, and is similar in peripheral resting T cells, in T cells activated for 2 days with concanavalin A (Con A) (Figs 2*a*, 4*b*), and in the immature double-negative thymocytes from 4-week-old adult mice (Figs 2*a*, 4*b*). The fact that our β probe preferentially detects $C_{\beta'}$ mRNA may explain why we fail to observe higher levels of β mRNA in thymocytes than in mature T cells, as has been reported by others[12,24]; recent studies suggest that 97% of β transcripts in murine thymocytes use C_β, although $C_{\beta'}$ is used in most functional T cells thus far examined[30].

Genomic analysis suggests that there is only a single C_α gene (A. Hayday, G. Tanigawa, D. Diamond, H.S. and S.T., unpublished). α mRNA is barely detectable at day 16 of development and increases in level thereafter, although even in adult thymocytes it is lower than in 2C cells (Figs 2*b*, 3*b*). The late appearance of α mRNA may explain recent data suggesting that before day 17 of fetal development, thymocytes do not express the α/β heterodimer on the cell surface[34]. The level of α mRNA in resting or activated mature T cells is approximately three times that in 2C cells. The level of α mRNA in immature double-negative adult thymocytes is ~2–3-fold lower than that in 2C cells, or 6–9-fold lower than in mature peripheral T cells (Figs 2*b*, 4*a*).

Sequences coresponding to the constant region of the γ gene (C_γ) are relatively abundant in day 16 fetal thymocytes (approximately half the level in 2C by Northern analysis) and decrease in level thereafter (Figs 2*c*, 3*c*). C_γ mRNA is nearly 10-fold less abundant in young adult thymocytes than in 2C cells. The level of C_γ mRNA in adult double-negative thymocytes is the highest of any cells we have examined, corresponding to about twice the level in 2C RNA (Figs 2*c*, 4*c*). In striking contrast, the level of C_γ mRNA in mature T cells is extremely low (Figs 2*c*, 4*c*). A longer exposure of the gel shown in Fig. 2*c* was required to reveal a weak band corresponding to C_γ mRNA in mature T cells (not shown); we estimate the level of C_γ mRNA in these T cells to be more than 20-fold lower than that in 2C cells. The level of C_γ mRNA in T cells activated for 2 days with Con A is also very low (Figs 2*c*, 4*c*). C_γ mRNA levels were also very low in BALB.B lymph node T cells (not shown).

In contrast to the α and β constant-region gene segments, which appear to use a larger number of different variable-region gene segments, a single γ-chain variable-region gene segment is transcribed in each CTL clone thus far examined[15,21]. Therefore, we have been able to analyse the regulation of V_γ mRNA expression in developing thymocytes. V_γ-containing mRNA was detected in day 15 fetal thymocyte RNA using the ribonuclease

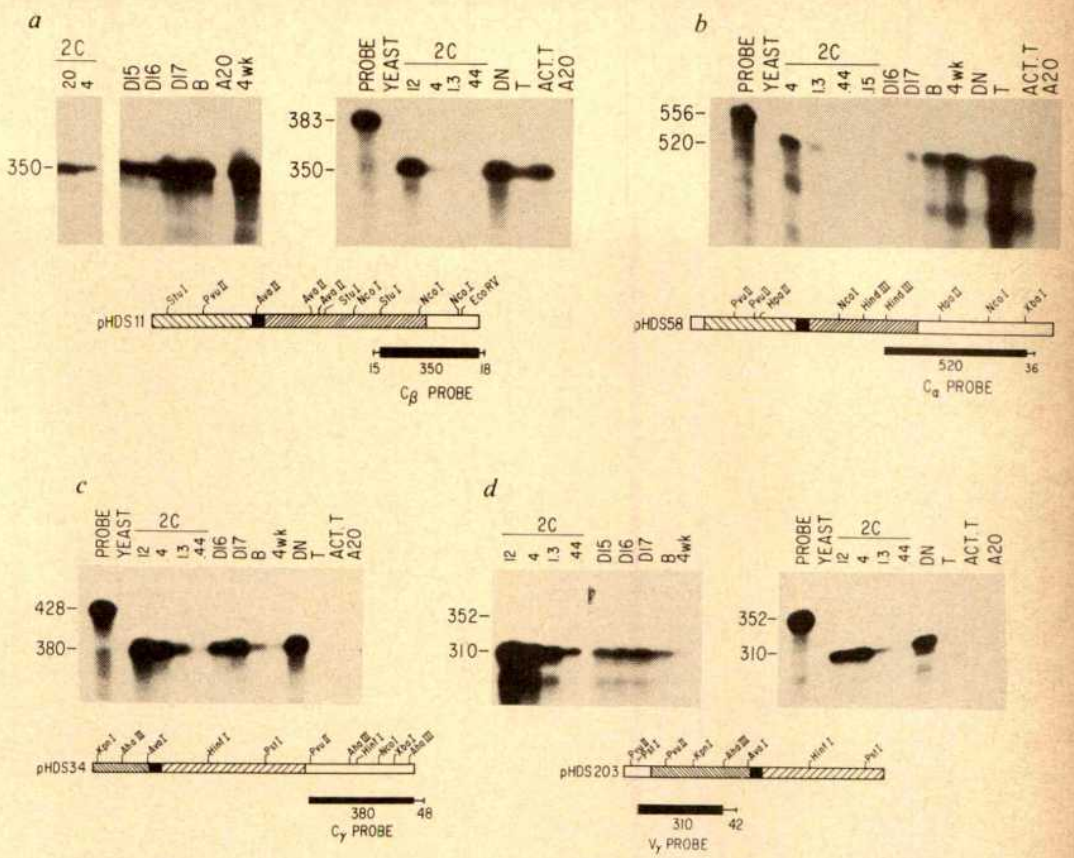


Fig. 2 Analysis of β (*a*), α (*b*), C_γ (*c*) and V_γ (*d*) mRNA levels in BALB/c AnN thymocyte and T-cell RNAs, using the ribonuclease protection assay[40]. 4 μg of total cellular RNA, or the indicated amounts of a standard preparation of total cellular RNA from the 2C cell line, were analysed. Samples containing <4 μg of 2C RNA were supplemented with yeast RNA. D15, D16 and D17 refer to RNAs prepared from thymocyte suspensions of fetuses killed 15, 16 or 17 days after detection of the vaginal plug (day 0). B, Newborn thymocytes (day 20 after detection of the vaginal plug); 4 wk, thymoctes from 4-week old mice; T, RNA from nylon-wool column-purified lymph-node T cells from 8-week-old mice, and ACT.T, RNA from the same cells activated for 42 h with Con A in the presence of equal numbers of irradiated splenic accessory cells; DN, RNA from 'double-negative' thymocytes purified from thymocyte suspensions from 4-week-old mice by two rounds of lysis with anti-L3T4 (GK1.5; ref. 41) and anti-Lyt-2 (AD4(15); ref. 42) monoclonal antibodies plus complement, and Ficoll–Isopaque purification of viable cells. Such cells are estimated to >90% pure double-negative thymocytes by cell sorter analysis (not shown). A20, RNA from the A20 B-lymphoma cell line. A20 RNA and yeast soluble RNA (4 μg) served as negative controls.

Methods. The probes were derived by subcloning in reverse orientation the indicated restriction fragment from pHDS 11 (β; ref. 15), pHDS 58 (α; ref. 17), pHDS 203 (V_γ; ref. 15) and pHDS 34 (C_γ) downstream from the SP6 promoter in the SP6 plasmids[40]. (pHDS 34 is a cDNA clone containing the 3′ portion of the γ gene; ref. 25.) ^{32}P-labelled single-stranded (SS) RNA probes complementary to T-cell receptor mRNAs were transcribed *in vitro* from the linearized SP6 plasmids, using the SP6 polymerase, in the presence of 10 μM ^{32}P-UTP (600 Ci mmol^{-1}) and excess cold ATP, GTP and CTP[40]. The lengths of the probes derived from the β, α and γ cDNAs are indicated in the diagrams (blacked in), as well as the length of the SP6 plasmid sequences also included in the transcripts (flanking lines). The ^{32}P-labelled probes were gel-purified and hybridized for 12 h at 40 °C with total cellular RNA, isolated by the guanidinium isothiocyanate/CsCl technique[43] from different cell types. The hybridization mixture was then treated with ribonuclease at 22 °C to digest single-stranded RNA. The ribonuclease was destroyed by incubation with proteinase K followed by phenol–chloroform extraction, and the RNA was ethanol-precipitated. The samples were electrophoresed on denaturing 7M urea/6% polyacrylamide gels, and the gels were subjected to autoradiography. Undigested probe was run on each gel; digestion of the SP6 plasmid-derived sequences from the probe in the experimental samples confirms the specificity of the assay.

protection assay, at ~10–15% the level found in clone 2C RNA (Fig. 2*d*). As the level of 1.5-kb C_γ mRNA in fetal thymocyte RNA is ~50% the level in 2C cells (see above), fetal thymocytes apparently contain 1.5-kb transcripts with C_γ sequences that do not hybridize with our V_γ probe. These transcripts may represent so-called 'sterile transcripts' of the γ gene, although sterile transcripts are usually shorter than their complete counterparts[24,32,33]. Alternatively, high-level transcription of C_γ rearranged to a distinct V_γ gene segment which does not hybridize with our V_γ probe would also account for the observed discrepancy.

V_γ mRNA declines to very low levels in adult thymocytes. Northern blot analysis confirmed that V_γ mRNA levels are low early in development compared with 2C and fall further after day 17 of fetal development (Fig. 3*d*). However, the level of V_γ mRNA in the adult double-negative thymocytes is much higher (approximately sevenfold) than that in fetal thymocytes (Fig. 2*d*). As is the case for C_γ-containing mRNAs, the level of V_γ mRNA in mature resting or activated T cells is extremely low, requiring for detection a longer exposure of the gel shown in Fig. 2*d*.

As the γ locus appears to undergo similar gene rearrangements on most rearranged chromosomes[15,25], Southern blot analysis of DNA from a T-cell population allows one to make a positive assessment of the extent of gene rearrangement. Rearrangement of C_γ gene segments is already detectable in DNA from fetal thymocytes at day 15, as evidenced by the appearance of a faint band of high relative molecular mass, and the diminution in intensity of the 13.4-kb germline band (Fig. 4*d*). The frequency of rearranged alleles increases during development, but ~50% of the alleles are still unrearranged even in adult thymocytes[25] (Fig. 4*d*). Our data suggest that the level of V_γ mRNA per rearranged γ allele prior to day 17 of fetal development is roughly comparable to the level in the 2C CTL line.

Approximately 50% of the γ alleles have undergone rearrangement in double-negative adult thymocytes, paralleling the increase in V_γ mRNA in these cells compared with fetal thymocytes (which also express the double-negative phenotype). These results suggest that the double-negative subpopulation has undergone maturation between the fetal and young adult stages with respect to rearrangement and expression of the γ genes. In contrast, mature T cells have a large proportion (~70%) of rearranged γ alleles[21] (Fig. 4*d*), yet have very low levels of γ mRNA.

Figure 1*b* shows the levels of α, β and γ mRNAs in thymocytes as a function of fetal age; the values given are rough estimates of the levels of 1.7-, 1.3- and 1.5-kb α, β and γ mRNAs respectively, relative to RNA from the 2C cell line, determined

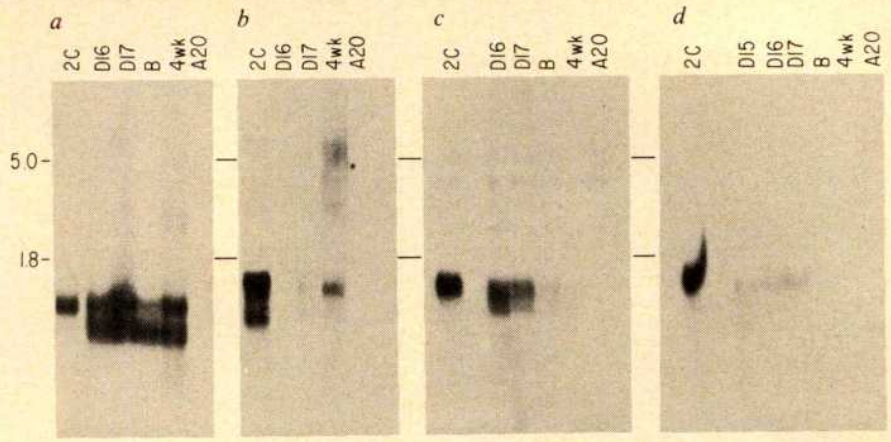


Fig. 3 Northern blot analysis of RNA samples from developing thymocytes. The probes are: *a*, C_β; *b*, C_α; *c*, C_γ; *d*, V_γ. The RNA samples are the same as those used in Fig. 2. The blots were hybridized with the same SP6-derived ^{32}P-labelled single-stranded probes as used in the ribonuclease assay (see Fig. 2 legend).

Methods. Total cellular RNA (5 μg) was denatured in formaldehyde, subjected to electrophoresis in 1.1% agarose/formaldehyde gels[43], then transferred to nitrocellulose filters, and hybridized with 3×10^7 d.p.m. of ^{32}P-labelled ssRNA probes(see Fig. 2 legend and probe diagrams) essentially as described by Melton *et al.*[40], except that the hybridized filters were washed at 70 °C rather than 65 °C in 0.1 × SSC, 0.1% SDS before autoradiography.

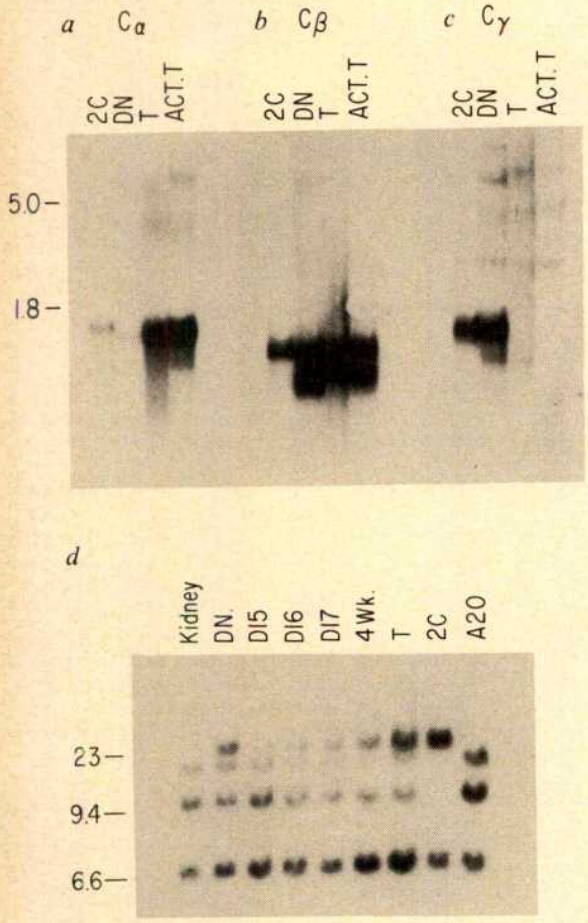


Fig. 4 *a–c*, Northern blot analysis of 5 μg of total cellular RNA from 'double-negative' adult thymocytes (DN), lymph-node T cells (T), activated T cells (ACT.T), and 2C CTLs (2C). The probes are *a*, C_α; *b*, C_β; *c*, C_γ. *d*, Rearrangements of the γ gene in thymocytes and T cells. Southern blot analysis[44] of *Eco*RI-digested genomic DNA from BALB/c kidneys, fetal thymocytes (D15, D16 and D17), 4-week-old thymocytes (4 wk) and purified lymph-node T cells from 8-week-old mice (T). DNAs from the A20 B-cell lymphoma (BALB/c-derived) and the 2C line (BALB.B-derived) were also included in the analysis. BALB/c and BALB.B embryo patterns are indistinguishable[21].

Methods. *a–c*, Northern blot analysis was performed as described in Fig. 3 legend. The preparation of the cells and RNAs were as described in Fig. 2 legend. *d*, Samples (5 μg) of each DNA preparation were digested with *Eco*RI, electrophoresed through 0.8% agarose gels, and transferred to nitrocellulose filters. The filters were hybridized with a ^{32}P-labelled, nick-translated probe corresponding to the constant region of the pHDS 34 γ cDNA clone. The probe extended from the *Ava*I site near the *J* region in pHDS 34 to the 3′ end (see Fig. 2*c*).

primarily from the Northern data (Figs 3, 4) with reference to the ribonuclease protection data (Fig. 2). The most striking feature of our results is that γ mRNA is relatively abundant only in the least mature cells of the T-cell lineage, whereas α mRNA is more abundant in mature than immature T cells. β mRNA is roughly equally abundant in each cell population examined. Whereas mRNA levels do not necessarily predict the absolute amount of the corresponding protein in a cell, the data presented here, which show shifts in the relative levels of mRNAs, probably reflect developmental regulation of the corresponding proteins.

What role might the γ gene play in T-cell recognition? Both class II-restricted helper T cells and class I-restricted CTL use a heterodimer of the α and β chains as at least part of their antigen/MHC receptor[6,7]. Although T-cell recognition is apparently not mediated by the independent recognition of MHC and antigen by independent receptors[35–37], other variations of the two-receptor model are still viable, particularly those which emphasize the constraints on physical interactions between H-2 molecules and antigens. Thus, the γ gene may encode part of a second T-cell receptor. However, based on recent evidence[38], it is unlikely that the specificity of either of the two putative receptors can be neatly assigned to either antigen or MHC determinants.

An alternative hypothesis is suggested by our data: perhaps the γ gene functions primarily in early T-cell differentiation. γ mRNA levels appear to be inversely related to α mRNA levels in thymocyte populations. Perhaps early in development a heterodimer of the γ and β chains is present on the surface of thymocytes prior to expression of the α gene. The putative γ/β heterodimer may serve as a receptor on immature thymocytes, allowing selection or suppression of clones expressing particular β chains. Later in thymocyte development, accompanying high-level expression of the α gene and a decrease in γ mRNA, the α-subunit may replace the γ subunit to form the receptor on mature T cells. It should be emphasized that the role of a γ-subunit in such a process need not be passive: for example, the γ chain may focus the γ/β-receptor complex onto MHC glycoproteins.

The finding that 2C and other CTL clones express relatively abundant levels of γ mRNA compared with normal T cells may result from the non-physiological growth conditions of the cells or, alternatively, induction of the γ gene in highly activated CTLs for purposes related to CTL function. However, the levels of γ mRNA are very low in T cells activated for 2 days with Con A (Figs 2, 4), which demonstrate significant CTL activity[39] (data not shown).

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1. Kindred, B. & Shreffler, D. C. *J. Immun.* **109**, 940-943 (1972).
2. Katz, D. H., Hamaoka, T., Dorf, M. E., Maurer, P. H. & Benacerraf, B. *J. exp. Med.* **138**, 734-739 (1973).
3. Zinkernagel. R. M. & Doherty, P. C. *Nature* **248**, 701-702 (1974).
4. Shearer, G. M. *Eur. J. Immun.* **4**, 527-533 (1974).
5. Allison, J. P., McIntyre, B. W. & Bloch, D. *J. Immun.* **129**, 2293-2300 (1982).
6. Haskins, K. *et al. J. exp. Med.* **157**, 1149-1169 (1983).
7. Meuer, S. C. *et al. J. exp. Med.* **157**, 705-719 (1983).
8. Bevan, M. J. *Nature* **269**, 417-419 (1977).
9. Zinkernagel, R. M. *et al. J. exp. Med.* **147**, 882-896 (1978).
10. Bevan, M. J. & Fink, P. J. *Immun. Rev.* **42**, 3-19 (1978).
11. von Boehmer, H., Haas, W. & Jerne, N. K. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2439-2442 (1978).
12. Yanagi, Y. *et al. Nature* **308**, 145-149 (1984).
13. Hedrick, S. M., Cohen, D. I., Nielson, E. A. & Davis, M. M. *Nature* **308**, 149-153 (1984).
14. Hedrick, S. M., Nielson, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153-158 (1984).
15. Saito, H. *et al. Nature* **309**, 757-762 (1984).
16. Chien, Y. *et al. Nature* **312**, 31-35 (1984).
17. Saito, H. *et al. Nature* **312**, 36-40 (1984).
18. Acuto, O. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 3851-3855 (1984).
19. Hannum, C. H., Kappler, J. W., Trowbridge, I. S., Marrack, P. & Freed, J. H. *Nature* **312**, 65-67 (1984).
20. Fabbi, M., Acuto, O., Smart, J. E. & Reinherz, E. L. *Nature* **312**, 269-271 (1984).
21. Chien, Y. *et al. Nature* **309**, 322-326 (1984).
22. Siu, G. *et al. Cell* **37**, 393-401 (1984).
23. Kavaler, J., Dialynas, D., Fitch, F. & MacDonald, H. R. *Nature* **310**, 421-423 (1984).
24. Siu, G. *et al. Nature* **311**, 344-349 (1984).
25. Kranz, D. M. *et al. Nature* **313**, 752-755 (1985).
26. Hayday, A. *et al. Cell* (in the press).
27. Ceredig, R., Dialynas, D., Fitch, F. & MacDonald, H. R. *J. exp. Med.* **158**, 1654-1671 (1983).
28. Fowlkes, B. J. thesis, George Washington Univ. Grad. School. of Arts and Sciences, Washington DC (1984).
29. Kranz, D. M., Sherman, D. H., Sitkovsky, M. V., Pasternack, M. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81**, 573-577 (1984).
30. Gascoigne, N. *et al. Nature* **310**, 387-391 (1984).
31. Malissen, M. *et al. Cell* **37**, 1101-1110 (1984).
32. Clark, S. P. *et al. Nature* **311**, 387-389 (1984).
33. Yoshikai, Y. *et al. Nature* **312**, 512-524 (1984).
34. Roehm, N. *et al. Cell* **38**, 577-584 (1984).
35. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. *J. exp. Med.* **153**, 1198-1214 (1981).
36. Hunig, T. R. & Bevan, M. J. *J. exp. Med.* **155**, 111-125 (1982).
37. Hever-Katz, E. *et al. J. exp. Med.* **155**, 1086-1099 (1982).
38. Marrack, P., Shimonkevitz, R., Hannum, C. H., Haskins, K. & Kappler, J. *J. exp. Med.* **158**, 1635-1646 (1983).
39. Bevan, M. J., Langman, R. E. & Cohn, M. *Eur. J. Immun.* **6**, 150 (1976).
40. Melton, D. *et al. Nucleic Acids Res.* **12**, 7035 (1984).
41. Dialynas, D. *et al. Immun. Rev.* **74**, 29-56 (1983).
42. Raulet, D. H., Gollieb, P. D. & Bevan, M. J. *J. Immun.* **125**, 1136-1143 (1980).
43. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning, a Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1983).
44. Southern, E. *J. molec. Biol.* **98**, 503-517 (1975).

Identification of antigen receptor-associated structures on murine T cells

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The specific antigen receptor on human and murine T lymphocytes is a heterodimer of relative molecular mass (M_r) 80,000–90,000 (80–90K) composed of two 40–50K disulphide-linked glycoprotein subunits[1–8]. Peptide map analysis of the α- and β-chains of receptor isolated from distinct tumour cell lines suggests the presence of both constant and variable regions[2,9–12]. Unlike the antigen receptor on B lymphocytes (that is, surface immunoglobulin), the human T-cell antigen receptor seems to be non-covalently associated with another invariant structure recognized by monoclonal antibodies to the cell-surface antigens T3 and Leu 4 (refs 4, 5, 9, 12). Meuer *et al.*[5] have demonstrated co-modulation of the T3 structure and T-cell antigen receptor using anti-clonotypic and anti-T3 monoclonal antibodies. Furthermore, immunoprecipitation with anti-T3 weakly co-precipitates a small amount of the 80–90K heterodimer in certain conditions[4,9,12]. The murine homologue of the Leu 4/T3 structure has not been identified, although Gunter *et al.* have suggested that Thy-1 may be the counterpart of Leu 4/T3 (ref. 13). Here we describe a Leu 4/T3-like structure, distinct from Thy-1, associated with the T-cell receptor of a murine T-lymphoma cell line.

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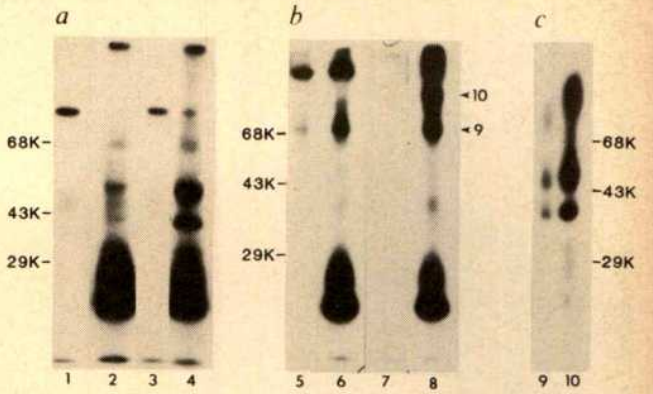


Fig. 1 SDS-PAGE analysis of human Leu 4 and associated structures. *a*, Reduced samples; *b*, non-reduced samples, run on 10% gels. Lanes 1, 2, 5, 6, sham-treated cells; lanes 3, 4, 7, 8, DTBP crosslinked cells; lanes 1, 3, 5, 7, P3 IgG1; lanes 2, 4, 6, 8, anti-Leu 4. *c*, Bands at arrows in *b* were cut out of dried gels, rehydrated in SDS sample buffer containing 50 mM dithiothreitol for 1 h, then loaded into sample wells and resolved on 12.5% gels.
Methods. Human HPB-ALL T-leukaemia cells (provided by Dr Jun Minowada) were labelled with ^{125}I (Amersham) using lactoperoxidase (Sigma)-catalysed radioiodination[14]. Half of the labelled cells were incubated for 30 min at room temperature (25 °C) in phosphate-buffered saline (PBS, 0.1 M phosphate, *p*H 7.5) containing 10 mM DTBP (Pierce Chemicals) as described by Wang and Richards[15], with minor modifications. Control cells were incubated in PBS without DTBP. After washing in 5 mM ammonium acetate/0.1 M PBS(*p*H 7.3) to inhibit the reaction, cells were placed in lysate buffer (0.05 M Tris-HCl, *p*H 8.0) containing 0.15 M NaCl, 1 mM EDTA (Sigma), 0.02% NaN, 0.5% Nonidet P-40 (Sigma), 20 KIU ml^{-1} aprotinin (Sigma) and 50 mM *N*-ethylmaleimide (Sigma) for 20 min at 4 °C. Lysates were centrifuged at 13,000*g* for 15 min, pre-cleared for 10 min with formalin-fixed *S. aureus* (Enzyme Center), then incubated at 4 °C with anti-Leu 4 antibody (Becton Dickinson Monoclonal Center) or P3X IgG1 myeloma protein. Immune complexes were isolated from lysates by incubation with rabbit anti-mouse IgG-coated formalin-fixed *S. aureus*. After washing, bound material was eluted by boiling in sample buffer containing 2.3% SDS (Bio-Rad) with or without 5% 2-mercaptoethanol (Sigma). Samples were analysed by SDS-PAGE as described by Laemmli[24]. Protein standards (Sigma) were included for M_r determination. Gels were dried and autoradiographed at −70 °C on Kodak XR film using intensifying screens (Cronex Lightning Plus).

The human T-cell antigen-specific receptor co-modulates on the cell surface with a 20–25K glycoprotein structure identified by the anti-T3 monoclonal antibody[5]. Immunoprecipitation of the T3 antigen from ^{125}I-labelled T-cell lines also weakly co-precipitates a small amount of the disulphide-linked 80–90K heterodimer under certain non-stringent conditions[4,9,12]. Here, we use chemical crosslinkers to perform a nearest-neighbour analysis of structures associated with human and murine T-cell receptor. We labelled the cell surface proteins of murine or human T cells with ^{125}I using lactoperoxidase-catalysed radioiodination[14], then crosslinked them using the cleavable reagent dimethyl dithiobispropionimidate (DTBP)[15]. The cells were solubilized with detergent and immunoprecipitates obtained with monoclonal antibodies and analysed by SDS-PAGE. Figure 1 shows such an analysis of human T-leukaemia HPB-ALL cells immunoprecipitated with an anti-Leu 4 antibody. Under reducing conditions (*a*), immunoprecipitates obtained from crosslinked cells contained significant amounts of two additional proteins of 37 and 45K in addition to the major Leu 4 bands at 22 and 27K. Lesser amounts of the 37 and 45K components were observed in the sham-treated controls. The properties of these proteins are consistent with the clonotypic heterodimer subunits described by Meuer *et al.*[4,5]. Immunoprecipitations of crosslinked and sham-treated samples with monoclonal antibodies to Leu 1 (T1), Leu 2 (TB), Leu 3 (T4) and Leu 5 (T11) failed to co-precipitate the 37 and 45K components, indicating

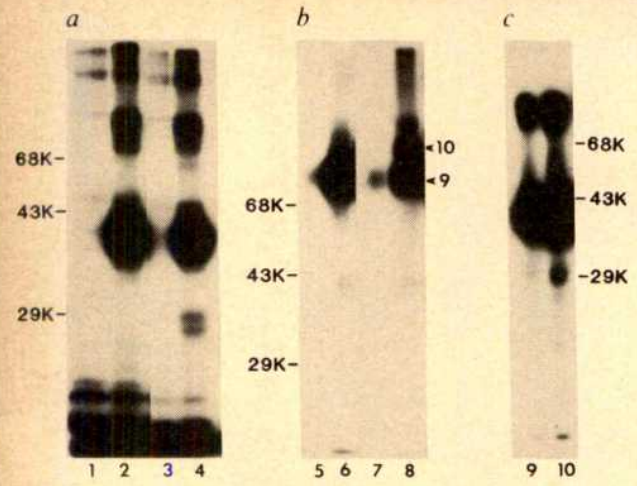


Fig. 2 SDS-PAGE analysis of T-cell receptor and associated structures from murine C6VL lymphoma cells. *a*, Reduced samples; *b*, non-reduced samples, resolved on 10% gels. Lanes 1, 2, 5, 6; sham-treated cells; lanes 3, 4, 7, 8, DTBP crosslinked cells; lanes 1, 3, 5, 7, P3 IgG1; lanes 2, 4, 6, 8, 124-40. *c*, Bands at arrows in *b* were cut out, rehydrated and reduced and contents resolved on 12.5% gels as described in Fig. 1 legend. C6VL lymphoma cells were radiolabelled, crosslinked and solubilized as described for Fig. 1. Crosslinked and sham-treated lysates were immunoprecipitated with the anti-T-cell receptor 124-40 antibody or P3X IgG1 myeloma protein.

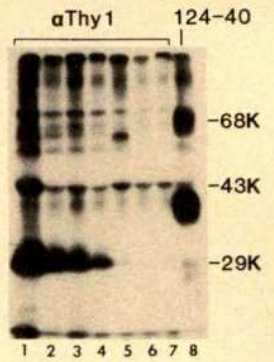


Fig. 3 Sequential immunoprecipitation with anti-Thy-1.2 and anti-T-cell receptor 124-40. Lanes 1–7, sequential immunoprecipitations with anti-Thy-1.2; lane 8, immunoprecipitation of supernatant with anti-receptor antibody 124-40. Thy-1.2 antigen was depleted from crosslinked C6VL lysate by precipitation with rat anit-mouse Thy-1.2 (clone 30-H12) antibody-coated *S. aureus*. After complete removal of all Thy-1.2 antigen, the depleted lysate was incubated with an anti-clonotypic antibody, 124-40, to the T-cell antigen receptor on C6VL, followed by rabbit anti-IgG/*S. aureus*. The bound material was eluted and analysed by SDS-PAGE under reducing conditions using a 10% polyacrylamide gel.

the specificity of the reaction (data not shown). That the additional bands were obtained as a consequence of crosslinking to the Leu 4 structure is supported by additional data. As shown in Fig. 1*b*, analysis of immunoprecipitates under non-reducing conditions revealed that both crosslinked and sham-treated cells yielded, in addition to the Leu 4 bands, an additional component at 83K, as expected for the T-cell receptor heterodimer co-precipitating as a result of non-covalent association with Leu 4. The crosslinked sample, but not the sham-treated sample, yielded an additional component of 117K, a size consistent with that expected for a covalent complex of the receptor heterodimer and Leu 4. This conclusion is supported by the data in Fig. 1*c*. Cleavage of the 83K component by reduction of the gel slice followed by SDS-PAGE indicated that this component was indeed composed of the receptor heterodimer subunits, whereas similar treatment of the 117K band yielded Leu 4-like components in addition to the receptor heterodimer subunits. Taken together, these data show that chemical crosslinking can demonstrate the association between Leu 4 and receptor-like components on the surface of human T cells.

We next sought to determine whether additional structures might be associated with the murine T-cell receptor heterodimer. We have described previously the properties of a murine anti-clonotypic monoclonal antibody 124-40, which reacts exclusively with the receptor of the murine T lymphoma C6VL[1,2]. This antibody immunoprecipitates a 80K glycoprotein composed of disulphide-linked subunits (39 and 41K)[1,2]. C6VL cells were radioiodinated, crosslinked with DTBP, solubilized and immunoprecipitated with antibody 124-40. As shown in Fig. 2, crosslinked, but not sham-treated, samples contain two additional components of 24 and 29K that co-precipitate with the T-cell specific heterodimer. These structures are not present in the non-specific antibody control. Anaylsis of the samples under non-reducing conditions (Fig. 2*b*) indicated the presence of the 80K receptor heterodimer, and, in the crosslinked sample only, an additional band of ~115K consistent with that expected for the receptor heterodimer and associated structures. As shown in Fig. 2*c*, reductive cleavage of the 115K band yields a band corresponding to the receptor subunits as well as additional components similar to those obtained in Fig. 2*a*. These results indicate that these structures may be a component of the murine T-cell antigen receptor complex similar to the Leu 4/T3 antigens that are associated with the human T-cell receptor heterodimer structure.

Recently, Gunter *et al.*[13] have proposed that murine Thy-1 may be the structural and functional counterpart to the human Leu 4/T3 antigen. This hypothesis was based on the observations that: (1) Leu 4/T3 and Thy-1 are present on all mature human and murine T lymphocytes, respectively[16–18]; (2) some monoclonal antibodies against both human Leu 4/T3 and murine Thy-1 are mitogenic[19–21]; (3) Leu 4/T3 and Thy-1 have a similar M_r (20–30K); (4) both anti-Leu 4 and UCHT-1 antibodies react with cerebellar Purkinje cells[22] (like the anti-brain reactivity of anti-Thy-1; ref. 16). However, unlike anti-Leu 4/T3 antibodies[18], G7 (anti-Thy-1) described by Gunter *et al.*[13] did not block specific T-cell antigen recognition, did not modulate readily the Thy-1 antigen on the cell surface of T lymphocytes and did not co-precipitate T-cell receptor heterodimer structures.

To address directly the relationship of the Thy-1 molecule and the two structures that co-precipitate with the T-cell receptor heterodimer from crosslinked C6VL membrane, we performed immunodepletion and peptide map analyses. As shown in Fig. 3, sequential immunoprecipitation with anti-Thy-1.2, which removed all Thy-1.2 antigen from the lysate, failed to remove the T-cell receptor heterodimer or the associated Leu 4/T3-like structures from crosslinked C6VL membrane lysates. These results indicate that the receptor is not associated with Thy-1 molecules bearing antibody-accessible 1.2 allotypic determinants. In view of the fact that the larger of the Leu 4/T3-like components was similar in electrophoretic mobility to Thy-1, it seemed possible that Thy-1 molecules, perhaps in a configuration resulting in the masking of the allotypic determinants, might comprise a portion of the receptor complex detected in the crosslinked precipitates. Therefore, we prepared peptide maps of each of the components for comparison of primary structure. The T3-like α-(29K) and β-(24K) components and Thy-1.2 were isolated from radioiodinated, crosslinked C6VL cells by immunoprecipitation with clonotypic antibody 124-40 or 30-H12, respectively, and purified by SDS-PAGE. Gel slices containing the proteins were subjected to partial proteolytic digestion and peptides resolved by SDS-PAGE as described previously[23]. Maps of peptides obtained with papain, *Staphylococcus aureus* V8 protease and subtilisin are presented in Fig. 4. The patterns obtained with 24K β-T3 are clearly different from the Thy-1.2 pattern with all three proteases. For the 29K



Fig. 4 Peptide map analysis of Thy-1.2 and the Leu 4/T3-like structures. *a*, Papain digests. Lanes 1, 4, 7, no protease; lanes 2, 5, 8, 5 μg papain; lanes 3, 6, 9, 25 μg papain. *b*, *S. aureus* V8 protease digests. Lanes 1, 3, 5, 5 μg protease digests; lanes 2, 4, 6, 25 μg protease. *c*, Subtilisin digests. Lanes 1, 3, 5, 10 μg subtilisin; lanes 2, 4, 6, 50 μg subtilisin. The Thy-1.2 and the two Leu 4/T3-structures associated with the T-cell antigen receptor on C6VL tumour were cut from gels and peptide map analaysis performed essentially as described by Cleveland *et al.*[23]. Peptides were resolved by SDS-PAGE on 15% gels.

α-T3, the overall patterns obtained with papain (Fig. 4*a*) and subtilisin (Fig. 4*c*) are similar to Thy-1.2, although the major fragments of Thy-1.2 are somewhat smaller. The patterns obtained with V8 protease (Fig. 4*b*), however, are quite distinct. These results indicate that the Leu 4/T3-like components are both different from Thy-1.2 in primary structure and represent distinct cell-surface components.

Our experiments demonstrate conclusively for the first time that in the mouse, as in man, additional components are associated with the heterodimer structure implicated as the T-cell antigen receptor. Further structural and functional studies of the murine equivalent of this Leu 4/T3-like structure await production of an antibody against this antigen, which will be facilitated greatly by crosslinking techniques in conjunction with anti-clonotypic monoclonal antibodies to obtain the structure for immunization.

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1. Allison, J. P., McIntyre, B. W. & Bloch, D. *J. Immun.* **129**, 2293–2300 (1982).
2. McIntyre, B. W. & Allison, J. P. *Cell* **34**, 739–746 (1983).
3. Samelson, L. E., Germain, R. N. & Schwartz, R. H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6972–6976 (1983).
4. Meuer, S. C. *et al. Nature* **303**, 808–810 (1983).
5. Meuer, S. C. *et al. J. exp. Med.* **157**, 705–719 (1983).
6. Kaye, J. & Janeway, C. A. Jr *J. exp. Med.* **159**, 1397–1412 (1984).
7. Haskins, K. *et al. J. exp. Med.* **157**, 1149–1169 (1983).
8. Kappler, J. *et al. Cell* **34**, 727–737 (1983).
9. Reinherz, E. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4104–4108 (1983).
10. Allison, J. P. *et al.* in *Regulation of the Immune System* (eds Sercarz, E., Cantor, H & Chess, L.) (Liss, New York, 1984).
11. Allison, J. P. *et al. Immun. Rev.* (in the press).
12. Acuto, O. *et al. J. exp. Med.* **158**, 1368–1373 (1983).
13. Gunter, K. C., Malek, T. R. & Shevach, E. M. *J. exp. Med.* **159**, 716–730 (1984).
14. Keski-Oja, J., Mosher, D. F. & Vaheri, A. *Biochem. biophys. Res. Commun.* **74**, 699–704 (1977).
15. Wang, K. & Richards, F. M. *J. biol. Chem.* **249**, 8005–8011 (1974).
16. Reif, A. & Allen, J. M. V. *J. exp. Med.* **120**, 413–433 (1964).
17. Raff, M. C. *Nature* **224**, 378–380 (1968).
18. Reinherz, E. L., Hussey, R. E. & Schlossman, S. F. *Eur. J. Immun.* **10**, 758–762 (1980).
19. van Wauwe, J. P., de Mey, J. R. & Goossens, J. G. *J. Immun.* **124**, 2708–2713 (1980).
20. Jones, B. & Janeway, C. A. Jr *Eur. J. Immun.* **11**, 584–592 (1981).
21. Maino, V. C., Norcross, M. A., Perkins, M. S. & Smith, R. T. *J. Immun.* **126**, 1829–1836 (1981).
22. Garson, J. A. *et al. Nature* **298**, 375–377 (1982).
23. Cleveland, D. W. *et al. J. biol. Chem.* **252**, 1102–1118 (1977).
24. Laemmli, U. K. *Nature* **227**, 680–685 (1970).

Direct evidence that reverse cholesterol transport is mediated by high-density lipoprotein in rabbit

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Mammalian cells obtain cholesterol for membrane synthesis mostly via the receptor-mediated endocytosis of low-density lipoprotein (LDL)[1]. Macrophages and vascular endothelium additionally have receptors that recognize certain modified forms of LDL (for example, acetyl-LDL)[2,3]. The process by which cholesterol returns from peripheral cells to hepatocytes (reverse cholesterol transport) has not been established; although tissue culture studies have favoured high-density lipoprotein (HDL) as the principal vehicle[4,5], the *in vivo* evidence for this is meagre. When cholesterol-loaded macrophages are incubated in medium containing plasma, cholesterol moves from the cells to HDL and is then esterified by lecithin/cholesterol acyltransferase[6]. The accumulation of cholesteryl esters in the particles increases their size and decreases their density; enrichment with apoprotein E (apo E) also occurs, producing a decrease in electrophoretic mobility[7,8]. We now report that similar changes occur in the circulating HDL of rabbits, when their peripheral tissues are loaded with cholesterol by intravenous (i.v.) injection of acetylated or native human LDL. This result suggests that HDL is involved in reverse cholesterol transport *in vivo*.

The experiments were carried out in six New Zealand White rabbits (*Oryctolagus cuniculus*; 3.2–4.7 kg), maintained on Sg1

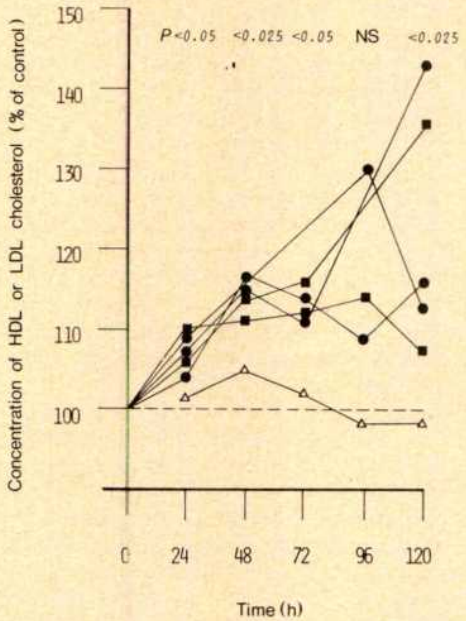


Fig. 1 Increases in the concentration of cholesterol in serum HDL measured by preparative ultracentrifugation (d = 1.063–1.21 g ml^{-1}) in five rabbits after a single i.v. injection (at time zero) of acetylated (●) or native (■) human LDL. The amount of cholesterol infused was 0.14–0.52 mmol (mean 0.29 mmol, 111 mg). The results are presented as percentages of the concentrations that existed at the time of injection. The P values at each time point were obtained by Student's paired t-test analysis of the absolute HDL cholesterol concentrations against the values at time zero. Mean concentrations of LDL cholesterol (d 1.019–1.063), in which there was no statistically significant change, are also given (△). The mean HDL cholesterol concentration (with the s.e.) at 0, 24 and 120 h was 0.88 (0.22), 0.94 (0.24) and 1.06 (0.24) mmol l^{-1} respectively; the corresponding values for LDL cholesterol were 0.79 (0.30), 0.68 (0.22) and 0.65 (0.20) mmol l^{-1}. Mean coefficient of variation of duplicate HDL cholesterol assays-3.8%.

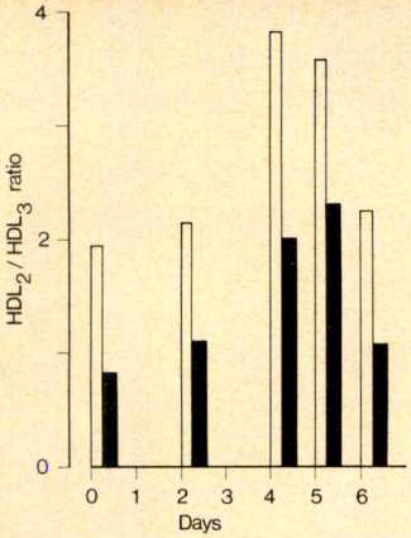


Fig. 2 Increase in the ratio of HDL_2 ($d = 1.063$–1.125 g ml^{-1}) to HDL_3 ($d = 1.125$–1.21 g ml^{-1}), measured in terms of total cholesterol content (open bars) and total protein content (closed bars) in a rabbit during the 6 days after a single i.v. injection of acetylated human LDL (0.49 mmol cholesterol, 189 mg). Results are the means of duplicate determinations.

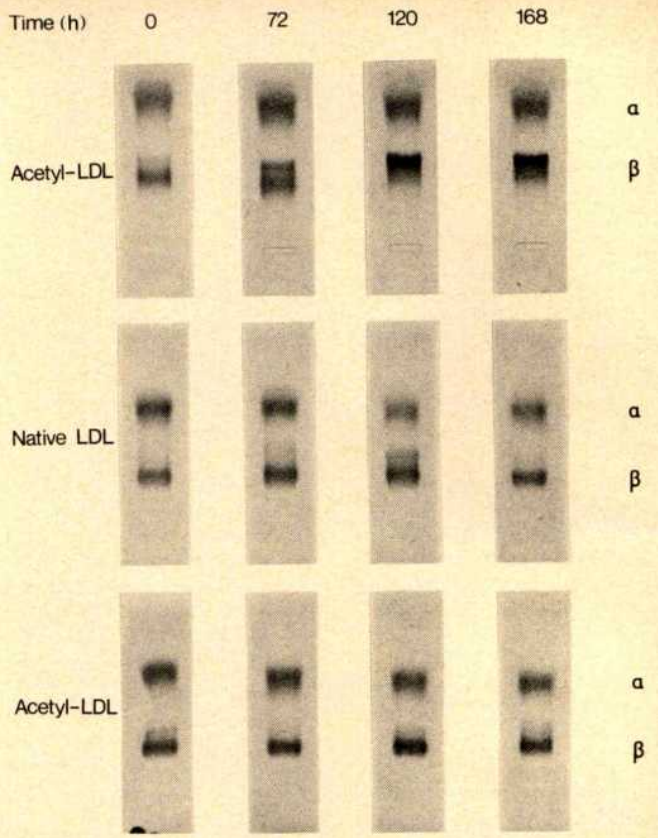


Fig. 3 Representative results from agarose gel electrophoresis of serum lipoproteins, using samples of serum collected immediately before, and at different times after, a single i.v. injection of acetylated or native human LDL into rabbits. (Serum samples were loaded at the bottom of the gels shown.) HDL migrates as the α band, and LDL as the β band. In all animals ($n = 5$) the mobility of HDL decreased progressively with time. In the pooled data, the mean electrophoretic mobilities of HDL (measured from the leading edge of the α band, and expressed relative to the pre-injection value) at 48, 72, 96, 120 and 168 h were (s.e. in parentheses): 98.5 (0.51), 96.6 (0.56), 94.0 (1.09), 91.0 (1.53) and 88.6 (1.86)%. By Student's paired t-test, the results obtained at all time points beyond 48 h were significantly different from those at time zero ($P < 0.05$ at 72, 96 and 120 h; $P < 0.02$ at 168 h). In some animals variable changes in the staining pattern of the β band were also observed; the significance of this finding, which was not associated with any change in LDL cholesterol concentration, is not clear, but one possibility is that it reflects the re-appearance in plasma of small amounts of modified LDL released from vascular cells by retroendocytosis[34].

rabbit diet (Grain Harvested Ltd, Canterbury, UK). LDL of density (d) 1.019–1.063 g ml^{-1} was isolated from human plasma by sequential preparative ultracentrifugation, dialysed against 0.15 M NaCl, and sterilized by filtration[9]. Acetyl-LDL was prepared using acetic anhydride, and agarose gel electrophoresis was performed to confirm that acetylation was complete[10]. Total cholesterol levels in serum LDL, very low-density lipoprotein (VLDL, $d < 1.006$), HDL (d 1.063–1.21) and the two major density subclasses of HDL (HDL_2, d 1.063–1.125; HDL_3, d 1.125–1.21) were determined by a cholesterol esterase/oxidase procedure (Boehringer-Mannheim, Cat. no. 237 574), after isolation of the lipoproteins by preparative ultracentrifugation[9]. In some animals, HDL cholesterol was also assayed after precipitation of VLDL and LDL with heparin and $MnCl_2$ (ref. 11). HDL protein was assayed according to Lowry *et al.*[12]. Electrophoresis of serum lipoproteins in agarose gel was performed for 30 min at 90 V (15 mA) using Corning Universal Agarose Film (1% agarose, 5% sucrose and 0.035% Na_2EDTA, (w/v) in 0.065 M barbital buffer, pH 8.6), followed by staining with 0.4% Fat Red 7B in methanol. Neutral and acidic steroids in faecal collections were quantified by gas–liquid chromatography, using 5α-cholestane as the internal standard[13,14].

Four animals were given acetylated LDL, and the remaining two native (unmodified) human LDL. All injections were made into an ear vein over 3–5 mins (total vol. 5–18 ml). The amounts infused (0.14–0.52 mmol cholesterol; mean 0.32) averaged 8.4 times the circulating LDL pool. As anticipated[15,16], both native and acetylated human LDL were cleared rapidly from the circulation, the plasma LDL cholesterol concentrations 24 h after injection being 79–150% (mean 101%) of initial values. The rationale behind giving acetylated and heterologous LDL, rather than the native homologous lipoprotein, was to target the cholesterol load away from hepatocytes and towards other cells. Although it was not possible to examine the tissue sites of uptake in our animals, it can be assumed from previous work that most of the acetyl-LDL will have been cleared by macrophages and vascular endothelium; these are the only cell types known to possess receptors with high affinity for acetyl-LDL[2,3,16,17], and acetyl-LDL does not bind to the native LDL receptors of other peripheral tissues and hepatocytes[2,17,18]. Accordingly, 10 min after injecting radioiodinated acetyl-LDL into rats, Van Berkel *et al.*[16] recovered 80% of the radioactivity in the non-parenchymal cells of the liver. Using ^{14}C-cholesteryl oleate-labelled acetyl-LDL, Blomhoff *et al.*[19] subsequently showed that this largely reflected uptake by vascular endothelium (60%), from which the cholesterol derived from hydrolysis of the lipoprotein was then released; uptake by hepatocytes was 15%. There also exists evidence that even unmodified human LDL is cleared largely by cells other than hepatocytes when injected into animals, although the organ sites of catabolism are distributed more widely than are those of acetyl-LDL[15,20–22]. Consistent with these reports was our own finding that the increments in faecal cholesterol and bile acid excretion during the 72 h after the injections accounted for <6% and <12% of the administered cholesterol in the case of acetyl-LDL and native LDL, respectively.

Following clearance of the infusate from the circulation, the plasma LDL concentration showed no significant change during the following 120–144 h (see Fig. 1 legend), nor did the level of VLDL cholesterol change. In contrast, HDL cholesterol, as measured by ultracentrifugation, progressively increased in all animals by a mean of 28% (range 14–43%; $P < 0.025$) (Fig. 1); this was accompanied by a rise in the HDL_2/HDL_3 ratio, measured in terms of both cholesterol and protein content (Fig. 2), indicating a decrease in the mean hydrated density of the particles. Concomitantly, the electrophoretic mobility of HDL decreased gradually in all animals (see Fig. 3).

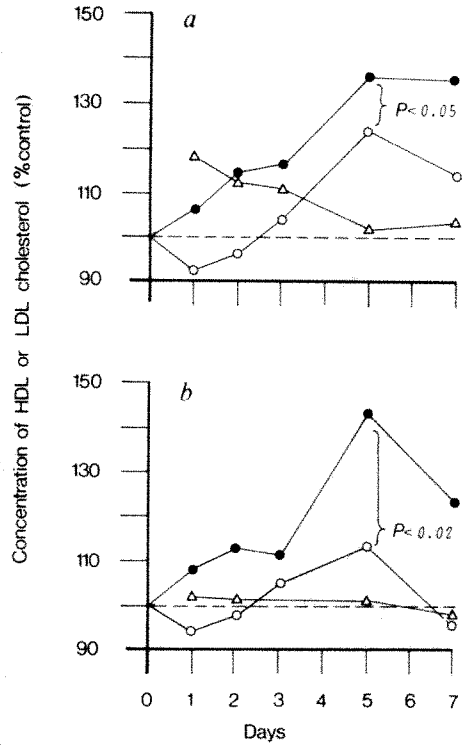


Fig. 4 Changes in the serum concentrations of HDL cholesterol (●, ○) and LDL cholesterol (△) in two rabbits, after i.v. injection of native (*a*) or acetylated (*b*) human LDL. The HDL values represented by the closed circles were obtained by ultracentrifugation ($d = 1.063$–1.21 g ml^{-1}); those represented by the open circles were obtained by assaying the cholesterol content of the supernatant after treatment of serum with heparin and $MnCl_2$ (ref. 11). LDL was isolated by ultracentrifugation (d 1.019–1.063). The P values were obtained by Student's paired t-test comparison of results obtained by the two different methods at the same time points. In the pooled data from four such experiments, the mean HDL cholesterol concentrations by ultracentrifugation at times 0, 72 and 120 h were 1.04 (0.19), 1.18 (0.22) and 1.26 (0.17) mmol l^{-1} respectively (s.e. in parentheses); the corresponding values determined using heparin-$MnCl_2$ were 0.98 (0.12), 1.01 (0.14) and 1.10 (0.17) mmol l^{-1}.

These changes in HDL are identical to those that occur in animals and man during consumption of cholesterol-rich diets[23] and in tissue culture when canine plasma is incubated with cholesterol-loaded mouse peritoneal macrophages[8]. In both cases there is accumulation of large HDL particles, rich in cholesteryl esters and apo E, which *in vitro* seem to be derived from HDL_3. As these particles differ functionally from HDL_3 in being no longer able to promote a net efflux of cholesterol from cultured cells[24], and in being catabolized in the perfused rat liver via high-affinity apo E receptors[25], it has been postulated that they represent the end-products of HDL metabolism during reverse cholesterol transport.

Apo E is synthesized in several tissues at a rate which is a function of cell cholesterol content[2,7,26]; it normally represents only a minor component of plasma total HDL, which can be divided into two subclasses on the basis of the presence or absence of the apoprotein. The results in Fig. 4 provide indirect evidence that the rise in total HDL cholesterol observed in our experiments was associated with a disproportionate increase in the apo E-containing subclass. HDL with apo E differs from HDL without apo E in being precipitable by heparin and $MnCl_2$ (ref. 23). When the concentrations of HDL cholesterol remaining in heparin-$MnCl_2$ supernatants were measured in four rabbits before and after injection of acetylated or native human LDL (two experiments with each), the relative increments were significantly smaller than those of total HDL cholesterol quantified by preparative ultracentrifugation.

Recently, the suggestion that HDL retards atherogenesis in man by removing cholesterol from the lesions[27] has received increasing attention as a result of epidemiological reports of an inverse relationship between the plasma HDL cholesterol concentration and the incidence of coronary heart disease[28,29]. Although the ability of HDL to remove cholesterol from cells is well documented *in vitro*[4,5], the *in vivo* evidence for such a role of HDL has been limited to demonstrations of a preferential transfer of radioactive cholesterol from tissues to plasma or lymph HDL in man[30,31], and to studies of the composition of peripheral lymph lipoproteins[32,33]. Our present finding that the delivery of cholesterol to peripheral tissues is followed by changes in HDL similar to those seen when the lipoprotein is incubated with cholesterol-loaded macrophages provides the first direct evidence that the events observed *in vitro* represent the normal process of reverse cholesterol transport, and suggests a model system for studying the efficiency of the process under different conditions *in vivo*.

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1. Goldstein, J. L. & Brown, M. S. *A. Rev. Biochem.* **46**, 897–930 (1977).
2. Brown, M. S. & Goldstein, J. *L. A. Rev. Biochem.* **52**, 223–261 (1983).
3. Stein, O. & Stein, Y. *Biochim. biophys. Acta* **620**, 631–635 (1980).
4. Glomset, J. A. *Am. J. clin. Nutr.* **23**, 1129–1136 (1972).
5. Nicoll, A., Miller, N. E. & Lewis, B. *Adv. Lipid Res.* **17**, 53–106 (1980).
6. Ho, Y. K., Brown, M. S. & Goldstein, J. L. *J. Lipid Res.* **21**, 391–398 (1980).
7. Basu, S. K., Goldstein, J. L. & Brown, M. S. *Science* **219**, 871–873 (1983).
8. Gordon, V., Innerarity, T. L. & Mahley, R. W. *J. biol. Chem.* **258**, 6202–6212 (1983).
9. Havel, R. J., Eder, H. A. & Bragdon, J. H. *J. clin. Invest.* **34**, 1345–1354 (1954).
10. Goldstein, J. L., Ho, Y. K., Basu, S. K. & Brown, M. S. *Proc. natn. Acad. Sci. U.S.A.* **76**, 333–337 (1979).
11. Warnick, G. R. & Albers, J. J. *High Density Lipoprotein Methodology Workshop* (ed. Lippel, K.) 53–69 (NIH publication no. 79-1661, Bethesda, Maryland, 1979).
12. Lowry, O. H., Rosebrough, N. J. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
13. Miettinen, T. A., Ahrens, E. H. & Grundy, S. M. *J. Lipid Res.* **6**, 411–424 (1965).
14. Grundy, S. M., Ahrens, E. H. & Miettinen, T. A. *J. Lipid Res.* **6**, 397–410 (1965).
15. Slater, H. R., Packard, C. J. & Shepherd, J. J. *J. biol. Chem.* **257**, 307–310 (1982).
16. Van Berkel, T. J. C., Nagelkerke, J. F., Harkes, L. & Kruijt, J. K. *Biochem. J.* **208**, 493–503 (1982).
17. Mahley, R. W. & Innerarity, T. L. *Biochim. biophys. Acta* **737**, 197–222 (1983).
18. Hass, A. J., Davis, H. R., Elner, V. M. & Glagov, S. *J. Histochem. Cytochem.* **31**, 1136–1138 (1983).
19. Blomhoff, R. *et al. J. biol. Chem.* **259**, 8898–8903 (1984).
20. Slater, H. R., Packard, C. J., Bicker, S. & Shepherd, J. *J. biol. Chem.* **255**, 10210–10213 (1980).
21. Koelz, H. R., Sherrill, B. C., Turley, S. D. & Dietschy, J. M. *J. biol. Chem.* **257**, 8061–8072 (1982).
22. Carew, T. E., Pittman, R. C. & Steinberg, D. *J. biol. Chem.* **257**, 8001–8008 (1982).
23. Mahley, R. W., Weisgraber, K. H., Bersot, T. P. & Innerarity, T. L. *High Density Lipoproteins and Atherosclerosis* (eds Gotto, A. M., Miller, N. E. & Oliver, M. F.) 149–176 (Elsevier, Amsterdam, 1978).
24. Daerr, W. H., Gianturco, S. M., Patsch, J. R., Smith, L. C. & Gotto, A. M. *Biochim. biophys. Acta* **619**, 287–301 (1980).
25. Sherrill, B. C., Innerarity, T. L. & Mahley, R. W. *J. biol. Chem.* **255**, 1804–1807 (1980).
26. Driscoll, D. M. & Getz, G. S. *Arteriosclerosis* **3**, 513a (1983).
27. Miller, G. J. & Miller, N. E. *Lancet* **i**, 16–19 (1975).
28. Miller, N. E., Forde, O. H., Thelle, D. S. & Mjos, O. D. *Lancet* **i**, 965–967 (1977).
29. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. & Dawber, T. R. *Am. J. Med.* **62**, 707–714 (1977).
30. Nestel, P. J. & Miller, N. E. *High Density Lipoproteins and Atherosclerosis* (eds Gotto, A. M., Miller, N. E. & Oliver, M. F.) 51–54 (Elsevier, Amsterdam, 1978).
31. Reichl, D., Myant, N. B., Rudra, D. N. & Pflug, J. J. *Atherosclerosis* **37**, 489–495 (1980).
32. Reichl, D., Rudra, D. N., Myant, N. B. & Pflug, J. J. *Atherosclerosis* **44**, 73–84 (1982).
33. Dory, L., Sloop, C. H., Boquet, L. M., Hamilton, R. L. & Roheim, P. S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3489–3493 (1983).
34. Aulinskas, T. H., Van der Westhuyzen, D. R., Bierman, E. L., Gevers, W. & Coetzee, G. A. *Biochim. biophys. Acta* **664**, 255–265 (1981).

Structure and transcription of human papillomavirus sequences in cervical carcinoma cells

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DNA of human papillomavirus (HPV) types 16 and 18 has been found closely associated with human genital cancer[1,2], supporting the concept that members of this virus group are key factors in the aetiology of genital cancer[3]. HPV 18 DNA sequences were also detected in cell lines derived from cervical cancer[2]. We have

now analysed these cell lines, HeLa, C4-1 and 756, for the structural organization and transcription of the HPV 18 genome and we find that the HPV 18 DNA is integrated into the cellular genome and is amplified in HeLa and 756 cells. Almost the complete HPV 18 genome seems to be present in 756 cells, with the early region being disrupted into two portions in each integrated copy. In HeLa and C4-1 cells, a 2–3 kilobase (kb) segment of HPV 18-specific sequences is missing from the E2 to L2 region. HPV 18 sequences are specifically transcribed from the E6–E7–E1 region into poly(A)$^+$ RNAs of 1.5–6.5 kb. Hybridization analysis of cDNA clones indicated that some of the transcripts are composed of HPV 18 and cellular sequences. In addition, poly(A)$^+$ RNA hybridizing with HPV 16 DNA was found in two out of three cervical carcinoma biopsies.

Two-dimensional agarose gel electrophoresis[4] and caesium chloride/ethidium bromide density gradient centrifugation[5,6], with subsequent analysis of the gradient fractions for the presence of HPV 18 sequences, showed that HPV 18 DNA is present in an integrated state within the chromosomal DNA of all three cervical cancer cell lines examined (data not shown). C4-1 cells were estimated to contain about one copy of HPV 18 DNA per cell, whereas in 756 and HeLa cells the HPV 18 sequences are amplified about 10–50-fold.

To determine which parts of the HPV 18 genome are present in the cell lines, blot hybridizations were performed using individual subgenomic fragments of HPV 18 DNA as nick-translated probes. Partial sequence analysis of prototype HPV 18 DNA and comparison with the known nucleotide sequence of the related genital papillomavirus HPV 6b (ref. 7, see Fig. 1*a*) allowed an unequivocal assignment of viral genome segments to each HPV 18 restriction fragment (Fig. 1*b*). The hybridization analysis then showed (Fig. 2) that all three cell lines contain (1) the HPV 18 non-coding region (hybridization of the cellular DNAs with the 1.1-kb *Bam*HI fragment) believed to harbour important regulatory elements for viral transcription (for review see ref. 8) and (2) the first part of the early region, including open reading frames E6, E7 and E1 (hybridization with the 2.4-kb *Bam*HI/EcoRI fragment). HeLa DNA seems to contain two different populations of HPV 18 sequences, one corresponding in its restriction enzyme cleavage sites (*Bam*HI, *Eco*RI) to the cloned HPV 18 prototype DNA and the other showing a modified pattern of cleavage sites (as seen by the hybridization of the 1.1-kb *Bam*HI fragment to two *Bam*HI fragments in HeLa DNA, Fig. 2*B*). Modifications in some HPV 18 cleavage sites were also observed in 756 (loss of the *Eco*RI site) and C4-1 DNA (data not shown). The lack of positive hybridization with the 1.4-kb *Hin*cII fragment (Fig. 2*B*) indicates the absence of HPV 18-specific sequences from the distal part of the early region (E2, E4) and from the late region (L2) in HeLa and C4-1 cells. In contrast, 756 cells seem to harbour most or even all of the HPV 18 DNA, as shown by hybridization of the 1.4-kb and 1.15-kb *Hin*cII fragments, respectively. The hybridization experiments suggest that HeLa and C4-1 cells contain only an incomplete HPV 18 genome with deletion of parts of the early and late region. However, we cannot rule out the possibility that these two cell lines contain the complete genome of a mosaic-like papillomavirus which, in the L1–E1 region, is composed of HPV 18-specific sequences and in the E2–L2 region is composed of sequences which do not cross-hybridize with HPV 18 in stringent conditions.

The hybridization data enabled us to deduce the possible locations of the viral integration sites. The 1.15-kb prototype HPV 18 fragment was the only probe that hybridized with two *Bam*HI fragments of 756 DNA (9 and 21 kb, respectively, Fig. 2*A*), indicating that opening of the circular HPV 18 DNA for integration has occurred in that region. Similarly, the hybridization patterns of HeLa and C4-1 DNA obtained with the 1.15-kb probe (Fig. 2*A*, *B*) suggest that one breakpoint of the integrated HPV 18 DNA is located downstream of the E6–E1 region, in the segment forming the 1.15-kb fragment, whereas in these two cell lines, because of the absence of some portions of HPV 18 sequences, the opposite viral integration sites are located in a late region segment covered by the 1.8-kb *Hin*cII/*Bam*HI fragment. Alternatively, in HeLa and C4-1 cells, the transition point between the HPV 18-related and unrelated sequences of a mosaic-like papillomavirus would be located in the 1.15-kb segment.

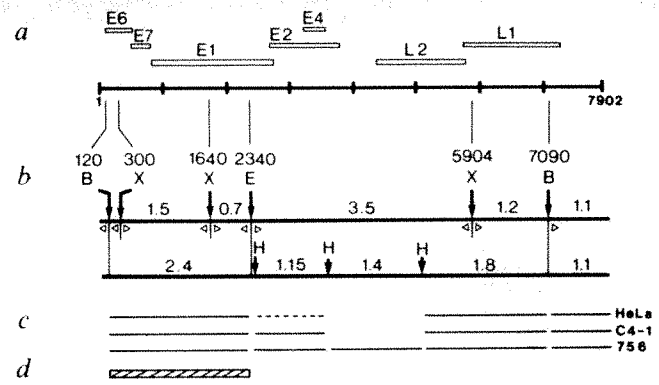


Fig. 1 ***a***, Arrangement of open reading frames in HPV 6b DNA as determined by nucleotide sequence analysis[7]. E and L denote open reading frames in the putative early and late regions, respectively. The circular 7,902-base pair (bp) HPV 6b sequence is linearized at nucleotide position 1 located in the non-coding region. ***b***, Alignment of HPV 18 DNA with HPV 6b according to nucleotide sequence homologies. Restriction fragments generated by cleavage with *Bam*HI (B), *Xba*I (X) and *Eco*RI (E) were used for partial sequence analysis of HPV 18 DNA (upper line). The fragment ends for which nucleotide sequence data were obtained (60–150 nucleotides from each 3′-terminally labelled end) are indicated by open triangles. 3′-Terminal labelling was performed in 50 mM Tris-HCl *p*H 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, with 50 μCi [α-^{32}P]dTTP (specific activity 3,000 Ci mmol^{-1}, Amersham) and 5 U DNA polymerase I (Klenow fragment, Boehringer) at 20 °C for 30 min. The partial chemical cleavage reactions for DNA sequencing were performed as described elsewhere[15]. The nucleotide positions in HPV 6b DNA homologous to the *Bam*HI, *Xba*I and *Eco*RI sites in HPV 18 DNA are indicated above the restriction sites. Isolated subgenomic fragments generated by cleavage of HPV 18 DNA with *Bam*HI, *Eco*RI and *Hin*cII (H, lower line) were used as nick-translated probes for hybridization with DNA and RNA from the HeLa, C4-1 and 756 cervical cancer cell lines. Sizes of restriction fragments are given in kb. ***c***, ***d***, Schematic summary of blot hybridization experiments performed with DNA (*c*) and poly(A)$^+$ RNA (*d*) from the three cell lines. HPV 18 fragments hybridizing to cellular DNA (see Fig. 2*A*, *B*) are represented by horizontal bars. The dashed line denotes very weak hybridization. The 2.4-kb *Bam*HI/*Eco*RI fragment found to be the only fragment hybridizing to poly(A)$^+$ RNA from HeLa, C4-1 and 756 cells (Fig. 3) is indicated by a cross-hatched bar.

Although the hybridization and mapping data are compatible with an overall co-linearity of the integrated HPV 18 sequences with the prototype HPV 18 DNA, they do not eliminate the possibility of sequence rearrangements that eventually may be reflected by some of the modifications in restriction enzyme cleavage sites observed in the integrated HPV 18 sequences. Figure 2C shows a scheme for the integrated HPV 18 DNA in the three cell lines. We assume that only one chromosomal integration site is present in C4-1 and 756 cells, respectively, but there is probably more than one in HeLa cells. The hybridization pattern of HeLa and 756 DNA further suggested that the amplified HPV 18 sequences are not arranged directly in tandem, but that the amplification includes flanking cellular sequences.

To analyse HPV 18 transcription in the three cell lines, poly(A)$^+$ RNA was isolated and subjected to Northern blot hybridization. Hybridization with total HPV 18 DNA as probe revealed for each cell line a characteristic RNA pattern showing two or three major RNA species (HeLa, 1.6 and 3.5 kb; 756, 1.5 and 6.5 kb; C4-1, 1.5, 4.2 and 5.5 kb). When the five subgenomic fragments of prototype HPV 18 DNA (Fig. 1*b*) were used as nick-translated probes, exactly the same RNA pattern was obtained with the 2.4-kb *Bam*HI/*Eco*RI fragment probe

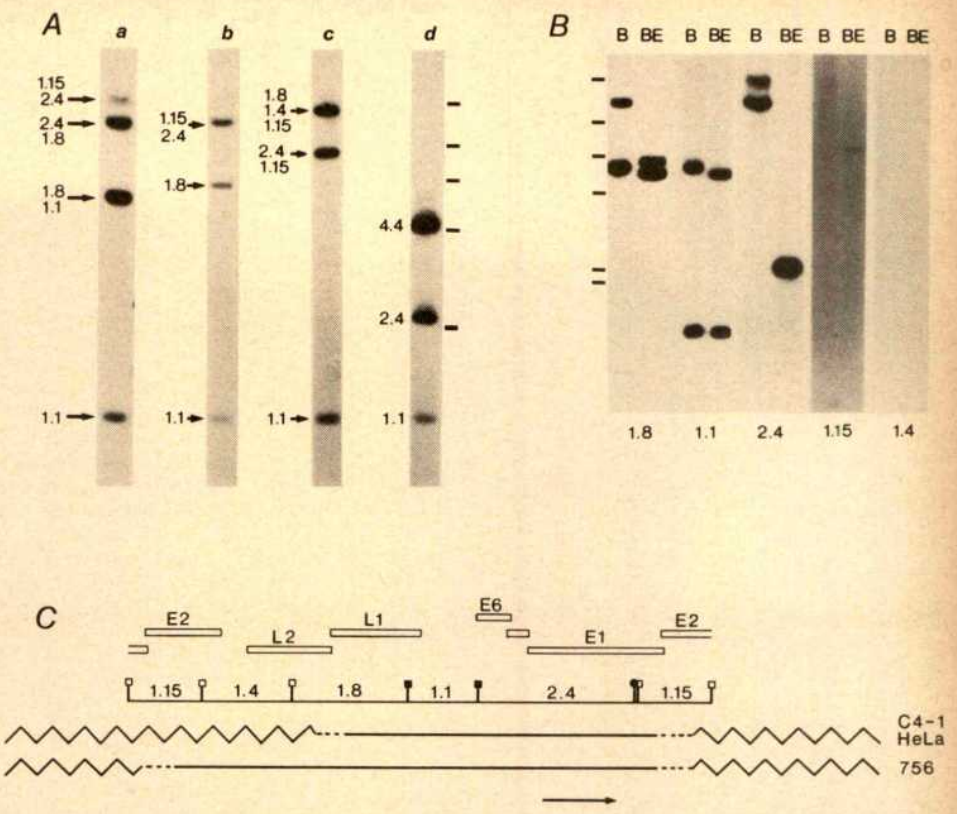


Fig. 2 *A*, Hybridization of DNA from human cervical cancer cell lines with HPV 18 DNA as probe. *B*, Hybridization of HeLa DNA with subgenomic fragments of HPV 18 DNA as probes. *C*, Possible integration patterns of HPV 18 DNA sequences into the cellular genome in HeLa, C4-1 and 756 cells as deduced from hybridization analysis with individual HPV 18 restriction fragments as probes. Cellular DNA sequences are represented by zigzag lines. Because the viral integration sites are not precisely known, the virus–cell junctions are indicated by dashed lines. A restriction endonuclease map of prototype HPV 18 DNA[2] is presented above, indicating the cleavage sites for *Hin*cII (□), *Bam*HI (■) and *Eco*RI (●). Sizes of restriction fragments are given in kb. The 1.15-kb *Hin*cII fragment is drawn at both sites of the linearized HPV 18 DNA. The arrangement of open reading frames in HPV 6b DNA is given on top. The arrow denotes the possible direction of transcription in HeLa cells (see text). Modifications in restriction enzyme cleavage sites in the integrated HPV 18 sequences are not indicated. The existence of at least two different populations of integrated HPV 18 sequences in HeLa cells is not taken into consideration. The integrated HPV 18 sequences as shown may contain putative sequence rearrangements which would disturb the strict co-linearity with the prototype HPV 18 DNA.

Methods. *A*, Cells of the HeLa line, C4-1 line[16] and 756 line[17], all established from human cervical cancer biopsies, were grown in monolayer cultures in Eagle's minimum essential medium containing 10% fetal calf serum. Cellular DNA was prepared from the nuclei of cells which were used to isolate cytoplasmic RNA by incubation in 1% sarcosyl, 25 mM Tris-HCl *p*H 8.0, 8.3 mM EDTA *p*H 8.0, 100 μg ml^{-1} proteinase K for 1 h at 37 °C. The DNA was purified by repeated extractions with phenol and chloroform: isoamyl alcohol (24:1) and was precipitated by the addition of isopropanol. Cellular DNA (10 μg) was cleaved with *Bam*HI, separated on a 0.8% agarose gel and transferred to nitrocellulose filter[18]: HeLa (lane *a*), C4-1 (lane *b*), 756 (lane *c*), 100 pg cloned HPV 18 DNA digested with *Bam*HI and *Eco*RI (lane *d*, the fragment sizes are indicated). HPV 18 DNA was purified from pBR322 vector DNA by agarose gel electrophoresis and was labelled by nick-translation[19]. Subgenomic fragments were prepared by digestion of HPV 18 DNA with *Bam*HI and *Eco*RI and fractionation of the cleavage products in a 0.8% agarose gel. Three fragments were isolated from the gel: 2.4-kb *Bam*HI/*Eco*RI, 1.1-kb *Bam*HI and 4.4-kb *Eco*RI/*Bam*HI fragment. The latter was further cleaved with *Hin*cII to prepare the 1.15-, 1.4- and 1.8-kb fragments (see Fig. 1*b*). Hybridizations were performed in 50% formamide, 5×SSC for 2 days at 42 °C. Filters were washed at 68 °C for 3×30 min in 2×SSC, 0.1% SDS and were exposed to X-ray films (Kodak X-Omat XR5) for 7 days with intensifying screens. Subgenomic fragments of HPV 18 DNA used as labelled specific probes (see Fig. 2*b* for HeLa DNA) are indicated by arrows at the respective cellular bands which they recognized. ***B***, HeLa DNA 10 μg was cleaved with either *Bam*HI (lanes B) or *Bam*HI plus *Eco*RI (lanes BE) and separated on 0.8% agarose gels. HPV 18 restriction fragments used as nick-translated probes are indicated at the bottom. The bars indicate the λ*Hin*dIII size marker fragments (23,130, 9,419, 6,557, 4,371, 2,322 and 2,028 bp). The results of the hybridization experiments are further schematically summarized in Fig. 1*c*.

(Fig. 3, lanes *a*–*d*). Apart from the 1.15-kb *Hin*cII fragment, which gave a faint hybridization signal for the 5.5-kb RNA of C4-1, no other nick-translated fragments hybridized to any RNA. The 2.4-kb fragment contains sequences homologous to positions 120–2,340 of HPV 6b DNA, thus representing the early open reading frames E6, E7 and most of E1 (Fig. 1). The Northern analysis therefore showed that in all three cell lines HPV 18 transcription is restricted to sequences from this part of the early region (Fig. 1*d*). The HPV 18-positive RNAs were estimated to comprise 0.01–0.02% of total poly(A)$^+$ RNA in 756 and C4-1 cells and ~0.2% in HeLa cells.

The orientation of the HPV 18 transcripts was analysed initially by using the 3′-terminally labelled strands of the 0.7-kb *Xba*I/*Eco*RI fragment (located within the E1 open reading frame; Fig. 1*b*) as single-stranded probes for Northern blot hybridization with HeLa poly(A)$^+$ RNA. The different amounts of label incorporated into the *Xba*I and *Eco*RI fragment ends, respectively, enabled us to distinguish the two DNA strands and to predict their polarity (Fig. 4 legend). The DNA strand containing the labelled 3′ *Xba*I end hybridized to the 3.5-kb RNA of HeLa, whereas the complementary strand containing the labelled 3′ *Eco*RI end failed to give any hybridization signal (Fig. 4), indicating that transcription proceeds in the sense direction through the early HPV 18 genes. The 1.6-kb RNA did not hybridize to either probe, suggesting that it does not contain sequences from the 0.7-kb segment.

The large size of some of the HPV 18-containing transcripts suggested that transcription may run further into flanking cellular (or non-HPV 18) sequences. Therefore, cDNA libraries from the poly(A)$^+$ RNA of the HeLa, 756 and C4-1 cell lines were constructed and cDNA clones positive for hybridization with HPV 18 DNA isolated (E.S. and Zs. Schwarz-Sommer, to be published elsewhere). Of 18 cDNA clones from 756 cells, 7 hybridized with nick-translated total DNA from human embryonic lung cells, indicating that they contain sequences which are repeated severalfold in the human genome. Furthermore, the genomic *Bam*HI fragment of 756 DNA harbouring the junction between the transcribed E6–E1 region of HPV 18 and the flanking cellular sequences also contains sequences in the cellular part which hybridize to nick-translated DNA of human embryonic lung cells (W.M., unpublished result). Together, these data strongly suggest that the seven 756 cDNA clones were derived from composite transcripts containing both early HPV 18 and cellular sequences. In contrast, all 40 HeLa and C4-1

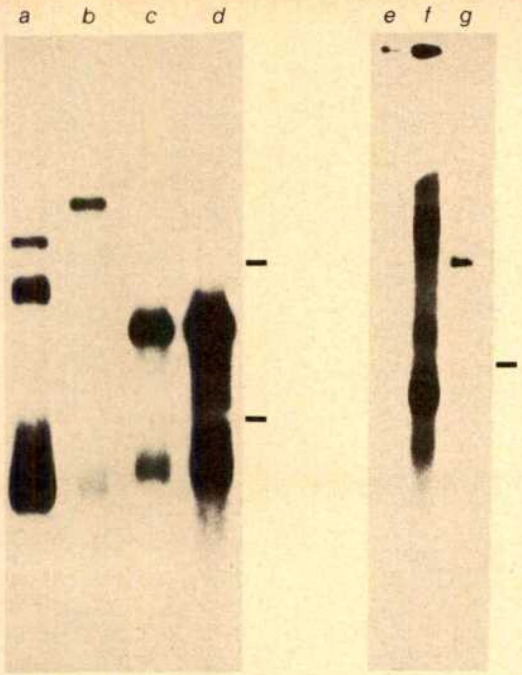


Fig. 3 Northern blot analysis of RNA from human cervical cancer cell lines (lanes *a*–*d*) and from cervical carcinoma biopsies (lanes *e*–*g*). Cytoplasmic RNA was prepared from tissue culture cells by lysis of cells in 0.6% NP40, 0.15 M NaCl, 10 mM Tris-HCl *p*H 7.4, 1 mM EDTA, pelleting of nuclei and repeated extractions of the supernatant with phenol–chloroform/isoamyl alcohol (24:1) as described elsewhere[20]. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography[21]. Total RNA from tumour biopsies was prepared essentially as described elsewhere[10] followed by isolation of poly(A)$^+$ RNA. RNA was fractionated on 0.8% formaldehyde agarose gels[22] and transferred to nitrocellulose filters, using 2 μg C4-1 (lane *a*), 1 μg 756 (lane *b*) and 0.1 μg HeLa (lane *c*) poly(A)$^+$ RNA. In lane *d*, 10 μg total cytoplasmic RNA from HeLa cells was fractionated. The 2.4-kb *Bam*HI/*Eco*RI fragment of HPV 18 DNA (see Fig. 1*b*) was used as nick-translated probe. The bars indicate the positions of 18S (1,850 bp) and 28S (4,850 bp) ribosomal RNA used as size markers. In lanes *e*–*g*, 5 μg poly(A)$^+$ RNA from each tumour biopsy were fractionated. HPV 16 DNA was used as hybridization probe which was purified from the vector DNA before nick-translation. The bar indicates the position of the 2-kb β-actin RNA identified by hybridization of the same filter with a nick-translated β-actin probe[23]. Hybridizations were performed in 50% formamide, 5×SSC for 48 h at 42 °C. Filters were washed in 2×SSC, 0.1% SDS at 68 °C and exposed to X-ray films for 2 days (lanes *a*–*d*) or for 7 days (*e*–*g*) with intensifying screens.

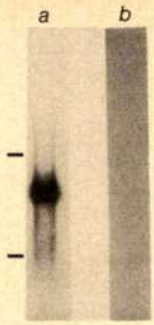


Fig. 4 Direction of HPV 18 transcription in HeLa cells. The 0.7-kb *Xba*I/*Eco*RI fragment of HPV 18 DNA (see Fig. 1*b*) was 3′-terminally labelled with DNA polymerase I (Klenow fragment) by incorporation of [α-^{32}P]dTTP. The DNA strands were separated in a 5% polyacrylamide gel in strand-separation conditions[15]. From the different amount of label incorporated (2 molecules TTP per *Eco*RI end and only 1 molecule per *Xba*I end), the more slowly migrating strand (1.2×10^6 c.p.m.) and the faster-migrating strand (2.3×10^6 c.p.m.) were deduced to contain the labelled 3′-*Xba*I end and 3′ *Eco*RI end, respectively. Northern blots with 1 μg HeLa poly(A)$^+$ RNA per slot were prepared and the filters were hybridized with the single-stranded DNA probes: lane *a*, slower-migrating strand labelled at the 3′ *Xba*I end; lane *b*, faster-migrating strand labelled at the 3′ *Eco*RI end. Autoradiography was for 15 days.

cDNA clones tested were negative in the hybridization with human embryonic lung cell DNA, indicating that they lack repeated cellular sequences.

Three cervical carcinoma biopsies examined were found to harbour HPV 16 DNA (and no HPV 18 DNA), but all differed in the amount of HPV 16 DNA present and in their *Bam*HI cleavage patterns (data not shown). Northern blot analysis using total HPV 16 DNA as probe revealed poly(A)$^+$ RNA species hybridizing with HPV 16 DNA in two of the samples (Fig. 3, lanes *e*-*g*). The RNAs transcribed varied greatly in amount and the major transcripts differed considerably in length. However, the small amount of DNA and RNA which could be isolated from the biopsies hampered further analysis.

The HPV 18-containing human cancer cell lines seem to differ from mouse cells transformed by bovine papillomavirus type 1 (BPV 1) and hamster tumours induced by BPV 1 in that the transcribed sequences are located in the E6–E7–E1 segment of the early region whereas the most abundant transcripts in BPV 1-transformed cells contain sequences from the end of the early region (E2, E4, E5)[9–11]. This part of the early region, however, is uncoupled from the upstream early sequences in 756 cells by integration of the HPV 18 DNA into the cellular genome and it is probably deleted in HeLa and C4-1 cells. Moreover, no transcripts from this region could be detected in 756 cells. Another difference is that the HPV 18 sequences are integrated into the host genome, a situation observed in most HPV 16- and HPV 18-positive cancer biopsies[2,24], whereas the BPV 1 genome persists mainly as an extrachromosomal episome (for review see ref. 12). In VX2 and VX7 transplantable carcinomas of domestic rabbits, however, DNA of the cottontail rabbit papillomavirus is integrated into the cellular DNA and the E6–E7 region is transcribed and spliced to the 3-terminal part of E2[13,14].

The transcription of specific early HPV 18 sequences in cervical cancer cell lines and the presence of HPV 16-positive poly(A)$^+$ RNA species in carcinoma biopsies further point to an active role of human papillomaviruses in genital cancer.

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1. Dürst, M., Gissmann, L., Ikenberg, H. & zur Hausen, H. *Proc. natn. Acad. Sci. U.S.A.* **80,** 3812–3815 (1983).
2. Boshart, M. *et al. EMBO J.* **3,** 1151–1157 (1984).
3. zur Hausen, H. *Curr. Topics Microbiol. Immun.* **78,** 1–30 (1977).
4. Wettstein, F. O. & Stevens, J. *Proc. natn. Acad. Sci. U.S.A.* **79,** 790–794 (1982).
5. Gissmann, L., de Villiers, E.-M. & zur Hausen, H. *Int. J. Cancer* **29,** 143–146 (1982).
6. Clewell, D. B. & Helinski, D. R. *Proc. natn. Acad. Sci. U.S.A.* **62,** 1159–1166 (1969).
7. Schwarz, E. *et al. EMBO J.* **2,** 2341–2348 (1983).
8. Gissmann, L. & Schwarz, E. in *Development in Molecular Virology* Vol. 5 (ed. Becker, Y.) 173–197 (Nijhoff, Hingham, Massachusetts, 1985).
9. Heilman, C. A., Engel, L., Lowy, D. R. & Howley, P. M. *Virology* **119,** 22–34 (1982).
10. Freese, U. K., Schulte, P. & Pfister, H. *Virology* **117,** 257–261 (1982).
11. Amtmann, E. & Sauer, G. *J. Virol.* **43,** 59–66 (1982).
12. Pfister, H. *Rev. Physiol. Biochem. Pharm.* **99,** 111–181 (1984).
13. Georges, E., Croissant, O., Bonneaud, N. & Orth, G. *J. Virol.* **51,** 530–538 (1984).
14. McVay, P., Fretz, M., Wettstein, F. O., Stevens. J. G. & Ito, Y. *J. gen. Virol.* **60,** 271–278 (1982).
15. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).
16. Auersperg, N. & Hawryluk. A. F. *J. natn. Cancer Inst.* **28,** 605–627 (1962).
17. Freedman, R. S. *et al. in vitro* **18,** 719–726 (1982).
18. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
19. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113,** 237–251 (1977).
20. Freese, U. K., Laux, G., Hudewentz, J., Schwarz, E. & Bornkamm, G. W. *J. Virol.* **48,** 731–743 (1983).
21. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69,** 1408–1413 (1972).
22. Seed, B. *Nucleic Acids Res.* **10,** 1799–1810 (1982).
23. Moos, M. & Gallwitz, D. *EMBO J.* **2,** 757–761 (1983).
24. Dürst, M., Kleinheinz, A., Hotz, M. & Gissmann, L. *J. gen. Virol.* (in the press).

Human genes for U2 small nuclear RNA map to a major adenovirus 12 modification site on chromosome 17

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U2 RNA is one of the abundant, highly conserved species of small nuclear RNA (snRNA) molecules implicated in RNA processing[1]. As is typical of mammalian snRNAs, human U1 and U2 are each encoded by a multigene family. In the human genome, defective copies of the genes (pseudogenes) far outnumber the authentic genes[2]. The majority or all of the 35 to 100 *bona fide* U1 genes[3–5] have at least 20 kilobases (kb) of nearly perfect 5′ and 3′ flanking homology in common with each other[4,6]; these U1 genes are clustered loosely in chromosome band 1p36 (refs 5, 7) with intergenic distances exceeding 44 kb. In contrast, the 10 to 20 U2 genes are clustered tightly in a virtually perfect tandem array which has a strict 6-kb repeating unit[8,9]. We report here the assignment, by *in situ* hybridization, of the U2 gene cluster to chromosome 17, bands q21–q22. Surprisingly, this region is one of three major adenovirus 12 modification sites which undergo chromosome decondensation ('uncoiling') in permissive human cells infected by highly oncogenic strains of adenovirus[10,11]. The two other major modification sites, 1p36 and 1q21, coincide with the locations of U1 genes[5,7] and class I U1 pseudogenes[12], respectively. We suggest that snRNA genes are the major targets of viral chromosome modification.

To map the U2 genes, we hybridized a U2 probe with the chromosomes of a normal female (Fig. 1*a–c*). In the 119 cells analysed, 164 (31.1%) of the total 528 silver grains were located over bands 17q12–q22, with a peak at q21. This result was confirmed by hybridizing the probe with the chromosomes of a normal male: in 39 cells, 34 (22.6%) of the total 168 grains lay over bands 17q12–q22. Figure 2 illustrates the combined data for chromosome 17 from the two experiments. Although there are many U2 pseudogenes in the human genome[2,13,14], no secondary sites of hybridization were detected in either experiment with normal human chromosomes (data not shown). However, most U2 pseudogenes are processed genes that contain only 33–82 bp of U2 sequence and lack flanking homology to the U2 genes[13]; *in situ* hybridization probably would not detect homologies of this length.

To delineate the region of assignment further, we hybridized the plasmid to cells carrying a human translocation chromosome. This chromosome, der(11), consists of the long arm of chromosome 17, from the middle of band q21 to the end of the long arm, translocated to the short arm of chromosome 11 [t(11; 17)(p15; q21)][15] (Fig. 3). Because cells from the human donor with the reciprocal translocation were no longer available, we used mouse–human hybrid cells retaining the der(11) chromosome under selective pressure[16]. The derivative chromosome was present at a frequency of 1.3 copies per cell. Of the 22 grains overlying this chromosome, 16 were localized over the junction region 11p15–17q22 (Figs 1*d*, 3). Thus, U2 genes are present in the region 17q21–q22.

The results obtained with the hybrid clone do not exclude the possibility that the U2 gene cluster was broken by the translocation in the parental human fibroblasts. The length of the U2 gene cluster (~100 kb), however, is small compared with that of a chromosome band (3,000–5,000 kb). Because the translocation breakpoint is near the middle of band 17q21, band 17q12 and the proximal portion of band q21 can reasonably be excluded as the site of the genes. In the normal lymphocytes a large proportion of the grains lay over band q21, with only a small percentage localized over q22. Therefore, the probable location of the genes is the distal half of q21.

The region 17q21–q22 containing the U2 genes is one of three major adenovirus type 12 modification sites[11]; productive infection of human cells causes these regions to appear 'uncoiled' and often to break[10]. Adenovirus types 2 and 5, which are not as highly oncogenic as type 12, also induce chromosomal gaps and breaks, but apparently at random sites[17]. Because the thymidine kinase (TK) gene maps near the modification site on chromosome 17 and adenovirus 12 infection stimulates TK production by a factor of 2–3 (ref. 18), McDougall[17] suggested that the localized alteration of chromosome structure might be related to virally induced transcription of specific host genes.

Previously, no genes had been correlated with the two other major modification sites on chromosome 1 in bands p36 and q21 (ref. 11). However, a minor modification site at 1q42 was localized immediately proximal to the 5S rRNA genes[19]. As shown in Fig. 4, the major modification site on the short arm of chromosome 1 coincides with the site of the U1 RNA genes in 1p36 (refs 5, 7). Further, the site in the long arm of chromosome 1, 1q21, is in the region 1q12–q22 to which we have assigned class I U1 RNA pseudogenes[12]. Pseudogenes of this

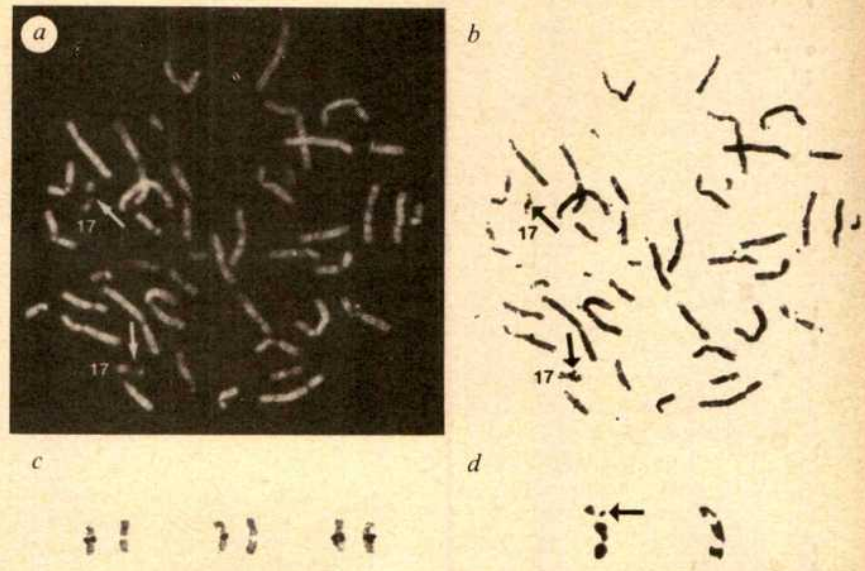


Fig. 1 Human metaphase chromosomes after *in situ* hybridization with the U2 probe. *a*, Representative chromosome spread from a methotrexate-synchronized culture of phytohaemagglutinin-stimulated lymphocytes[24], from a normal individual, stained with quinacrine mustard dihydrochloride[25,26]. Arrows indicate grains overlying the region 17q12–22. *b*, Cell shown in *a* after staining with 0.06% Wright stain. *c*, Pairs of chromosomes 17 from cells with grains overlying one or both homologues. *d*, der(11) chromosome, the result of the translocation t(11; 17)(p15; q21)[15], with typical labelling over the arm containing bands 17q21–qter (arrow). On the right, for comparison, is a trypsin-banded example of the chromosome. The U2 probe, pBR322 derivative pML2d with an 896-base pair (bp) insert containing the entire 188-bp U2 coding sequence, hybridizes only to the U2 array in Southern genomic blotting experiments (data not shown). The plasmid was labelled by nick-translation with three tritiated nucleotides (dATP, dCTP and dTTP) to a specific activity of 2.5×10^7 c.p.m. per μg of DNA. Hybridizations were carried out for ~16 h at 37 °C at a probe concentration of 25 ng ml^{-1} as described elsewhere[27]. Slides were coated with emulsion and developed after 5 days of exposure at 4 °C.

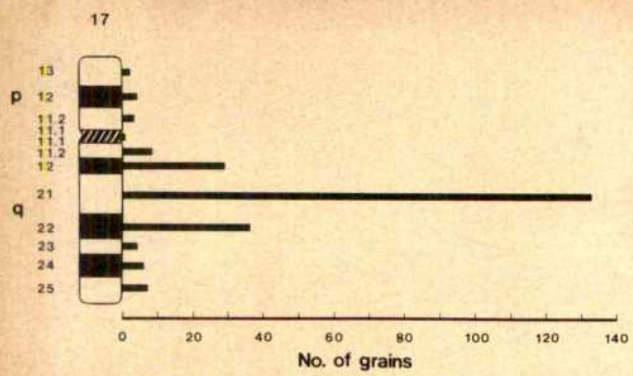


Fig. 2 Representation of chromosome 17, showing the distribution of grains after hybridization of the U2 probe with normal chromosomes from two human donors. Horizontal bars indicate the numbers of grains observed over the corresponding band in 158 metaphase spreads.

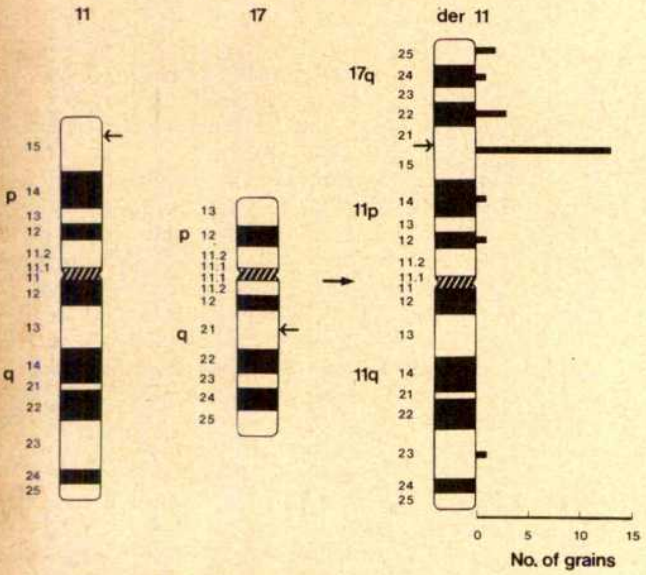


Fig. 3 *In situ* hybridization of the U2 probe with the der(11) chromosome in hybrid cells. Small arrows denote the breakpoints in bands 11p15 and 17q21 that led to the translocation t(11; 17)(p15; q21)[15] in the human parental fibroblasts. Hybrids between these fibroblasts and mouse thymidine kinase(TK)-deficient 3T3 cells were selected in hypoxanthine-aminopterin-thymidine medium to retain the der(11) chromosome, containing the region 17q21-qter, and/or the normal chromosome 17, both of which carry the human TK gene[16]. Bars next to the der(11) ideogram illustrate the numbers and positions of grains observed over 31 der(11) chromosomes in 24 hybrid cells. The peak of the grain distribution coincides with the lightly staining fusion band composed of 11p15 and 17q21.

class, unlike those of classes II and III, share extensive flanking homology with the true U1 genes[3,6]. All four well-characterized class I U1 pseudogenes that we have mapped to 1q12-q22 cross-react with the true U1 genes at 1p36 but with no additional chromosomal sites. Thus, most or all class I U1 pseudogenes map to the third modification site. As all three of the major adenovirus 12 modification sites contain snRNA genes or very closely related pseudogenes, and one of the minor sites is near the 5S rRNA genes, we propose that these highly transcribed genes are the primary targets of the viral modification.

Studies on the structure of active chromatin lead most naturally to the hypothesis that virally induced chromosomal unfolding reflects snRNA gene activation, although gene inactivation or localized DNA replication cannot be excluded. For example, the adenovirus E1a transforming gene product may

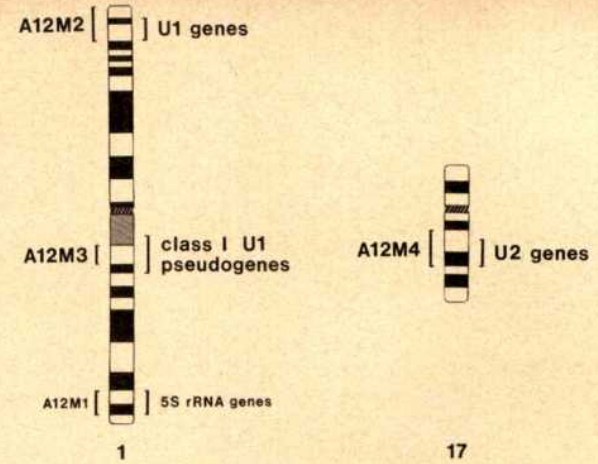


Fig. 4 Locations of human snRNA gene and pseudogene clusters correlate with the major adenovirus type 12 modification sites. As indicated by brackets, the three major viral modification sites, labelled A12M2, 3 and 4 (ref. 28), correspond exactly to the map positions of the U1 genes[5,7], class I U1 pseudogenes[12], and the U2 gene cluster, respectively. One of the minor modification sites, A12M1, is localized near the 5S rRNA genes[19].

activate the U1 and U2 genes in *trans*, as it does a variety of other endogenous[20] and exogenous[21,22] RNA polymerase II transcription units, as well as RNA polymerase III transcription units like the 5S RNA genes (A. J. Berk, personal communication). Alternatively, the E1a product may repress snRNA expression, as it does the expression of the class I major histocompatibility antigen in infected cells[23]. Class I U1 pseudogenes may be affected because they possess good flanking homology to the true U1 genes and may retain functional regulatory sequences in the DNA. An examination of the effects of viral infection on expression of host cell snRNA and of 5S rRNA may help to clarify the role of localized chromosomal uncoiling in the adenovirus life cycle.

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1. Busch, H., Reddy, R., Rothblum, L. & Choi, Y. C. *A. Rev. Biochem.* **51,** 617-654 (1982).
2. Denison, R. A., Van Arsdell, S. W., Bernstein, L. B. & Weiner, A. M. *Proc. natn. Acad. Sci. U.S.A.* **78,** 810-814 (1981).
3. Denison, R. A. & Weiner, A. M. *Molec. cell. Biol.* **2,** 815-828 (1982).
4. Manser, T. & Gesteland, R. F. *Cell* **29,** 257-264 (1982).
5. Lund, E. *et al. Molec. cell. Biol.* **3,** 2211-2220 (1983).
6. Bernstein, L. B., Manser, T. & Weiner, A. M. *Molec. cell. Biol.* (submitted).
7. Naylor, S. L., Zabel, B. U., Manser, T., Gesteland, R. & Sakaguchi, A. Y. *Somat. Cell molec. Genet.* **10,** 307-313 (1984).
8. Van Arsdell, S. W. & Weiner, A. M. *Molec. cell. Biol.* **4,** 492-499 (1984).
9. Westin, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **81,** 3811-3815 (1984).
10. zur Hausen, H. *J. Virol.* **1,** 1174-1185 (1967).
11. McDougall, J. K. *Cytogenet. Cell Genet.* **25,** 183 (1979).
12. Lindgren, V., Bernstein, L. B., Weiner, A. M. & Francke, U. *Molec. cell. Biol.* (submitted).
13. Van Arsdell, S. W. & Weiner, A. M. *Nucleic Acids Res.* **12,** 1463-1471 (1984).
14. Westin, G., Monstein, H.-J., Zabielski, J., Philipson, L. & Pettersson, U. *Nucleic Acids Res.* **9,** 6323-6338 (1981).
15. Francke, U. *Am. J. hum. Genet.* **24,** 189-213 (1972).
16. Francke, U. & Busby, N. *Cytogenet. Cell Genet.* **14,** 313-319 (1975).
17. McDougall, J. K. *J. gen. Virol.* **12,** 43-51 (1971).
18. Pina, M. & Green, M. *Virology* **38,** 573-586 (1969).
19. Steffensen, D. M., Szabo, P. & McDougall, J. K. *Expl Cell Res.* **100,** 436-439 (1976).
20. Kao, H.-F. & Nevins, J. R. *Molec. cell. Biol.* **3,** 2058-2065 (1983).
21. Green, M. R., Treisman, R. & Maniatis, T. *Cell* **35,** 137-148 (1983).
22. Gaynor, R. B., Hillman, D. & Berk, A. J. *Proc. natn. Acad. Sci. U.S.A.* **81,** 1193-1197 (1984).
23. Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. J. *Nature* **305,** 771-775 (1983).
24. Francke, U. & Oliver, N. *Hum. Genet.* **45,** 137-165 (1978).
25. Lawrie, S. S. & Gosden, J. R. *Hum. Genet.* **53,** 371-373 (1980).
26. Kirsch, I. R., Morton, C. C., Nakahara, K. & Leder, P. *Science* **216,** 301-303 (1982).
27. Harper, M. E. & Saunders, G. F. *Chromosoma* **83,** 431-439 (1981).
28. Shows, T. B., McAlpine, P. J. & Miller, R. L. *Cytogenet. Cell Genet.* **37,** 344 (1984).

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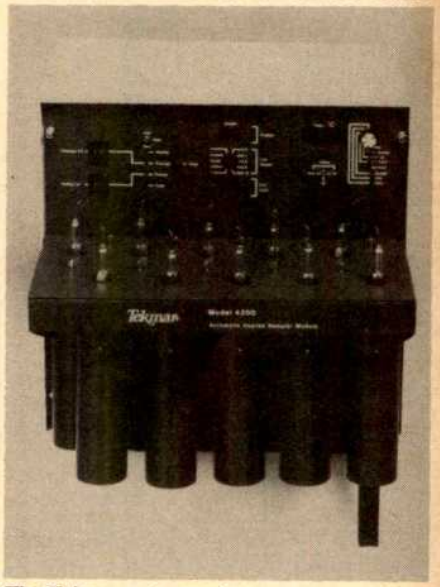
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from Richard Pearson

The involvement of prospective employers in sponsoring students in higher education can lead to improvement in courses in general, as well as benefiting individuals.

IN the 1980s, teachers and educationalists are expected to consider 'market signals' when planning course provision and curricula. Likewise students entering higher education are expected to adjust their subject preferences by noting changes in relative salaries, career prospects and unemployment rates. The hoped-for outcome is that educational output becomes more closely aligned to economic and labour market needs. As well as offering improved career prospects and higher salaries, employers are increasingly using sponsorship of students in higher education as a means of attracting those with scarce skills (*Nature* **307**, 488; 1984). To aid this process, in 1984 the UK Department of Education and Science increased the amount a student could receive from an employer by way of a term-time bursary from £915 to £1,600 a year, with the secretary of state commenting "I am sure that these increased sponsorship levels will help to attract able students to pursue courses of great importance both to them, to employers, and to the economy..."

For individual employers, the prime motive in sponsoring undergraduates is to meet their own recruitment needs. They are now spending over £10 million each year on such activities. In 1984, one in four final-year engineering undergraduates was being sponsored, while the number of final-year electronics graduates receiving sponsorship increased by over 70 per cent in the past five years. A report shortly to be published by the Institute of Manpower Studies (see Table 1) examines the extent to which this market signal is also affecting the subject choices of those entering higher education and thus expanding the overall supply of students in poorly patronized subjects.

There was clear evidence that the number of engineering students wanting sponsorship was far greater than the number of sponsorship places available. Given the real and perceived benefits of employer sponsorship — guaranteed industrial training, extra income, close links with an employer, and the probable offer of a job on graduation — this was not surprising (Table 1). The offer of sponsorship affected one in four students' choice of course and institution (Table 2). The biggest impact was on the choice of institution, although a significant minority switched to sandwich courses as a result of sponsorship. Of those switching subject, for a few the shift was fairly dramatic, for example from biochemistry to mechanical engineering, but for many it meant a more limited adjustment.

Table 1 Students' views on the benefits of sponsorship

Extra money	55%
Practical experience	41%
Future job prospects	29%
Vacation job	24%
Better training	14%
Other	5%

Percentages are proportions of sponsored students only. These data, and data in Table 2, are from "Employers Sponsorship of Undergraduate Engineers, by A. Gordon, R. Hutt & R. Pearson (Gower, Aldershot, in the press).

The impact on students depended to some extent on the way in which the employers' lists of preferred courses were drawn up. The criteria for inclusion were not only the perceived relevance or reputation of the course, but also the apparent status of the institution, the kind of student believed to be attracted to and accepted by it, the historical experience of senior managers and the recent experience of graduate recruitment personnel. Only a minority of employers included polytechnics on their preferred list.

Sponsorship can however cause difficulties for those not directly involved. Thus non-sponsored students on sandwich courses were finding it increasingly difficult to find training placements. Some teaching staff were concerned that links with a single employer might also make a student's industrial experience excessively narrow and in some cases unduly influence course content and pattern. The view was also expressed that because sponsorship focuses student and employer attention on certain, usually well known, institutions and courses, it can inhibit the development of new courses.

Because of sponsorship arrangements, each year a significant proportion of engineering graduates move directly into jobs with their sponsoring employer and effectively do not become job seekers in the annual graduate recruitment round. As a result, the number of newly qualified graduates actively seeking jobs in the labour market on graduation is considerably lower than the number graduating each year. Thus in 1983, some 500 or more newly graduating electrical/electronics engineers took jobs with their sponsoring employer while a further 600 to 800 chose to go on to research or further training or take jobs outside engineering. This meant that employers seeking to fill direct-entry appointments were recruiting from a pool of active job-seekers totalling some 2,000 rather than 3,100 newly graduating electrical/electronics engineers.

The sponsored graduates withdrawn from the open market in this way were not evenly distributed. They tended to be among the most highly qualified young engineers in terms of course entry qualifications, and were frequently concentrated in some of the most industrially oriented and often more prestigious departments and institutions. They had also often received relevant industrial training. Non-sponsoring employers seeking to recruit from these courses, and wanting a spread of ability and educational background in their new graduate recruits, will therefore have had increased difficulties.

Table 2 Influence of sponsorship on students' choices

Subject choice	14%
Type of course	25%
Institution	22%

Percentages refer to sponsored students only.

Overall it is clear that the availability of sponsorship has led directly to some students switching subjects and courses, a significant proportion in the case of production engineering, and acted as a positive signal to potential students about the subjects and courses employers value most. Sponsorship thus serves to increase, albeit by a small amount, the pool of students seeking to study in disciplines of the highest relevance to employers.

The content and pattern of degree courses is also being affected through the involvement of sponsoring employers, increasing their relevance to that group of employers. If these are also the most far-sighted and thoughtful employers in relation to engineering education, then the industry at large can be expected to benefit. However, sponsorship has most relevance and impact on market share, and on the quality of graduates recruited, aiding primarily the sponsoring employer and those directly involved, and has less impact on market size and the overall pool of engineering talent in the economy. □

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nature

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How not to fix the farm economy

President Reagan hopes the deficit will persuade Congress that farm programmes cannot be afforded. But a free market and exports will not cure all ills.

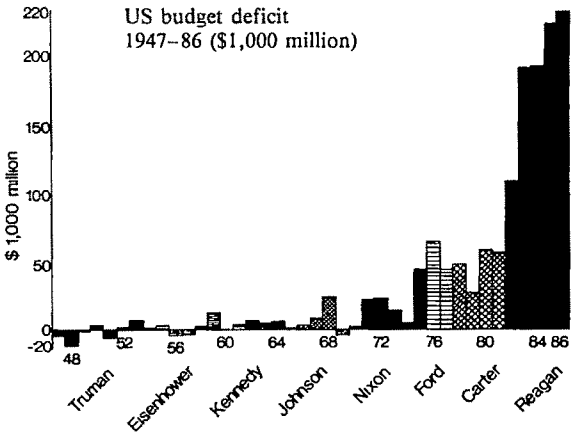
IRONY abounded last week as President Reagan vetoed legislation passed by Congress — with the help of his own Republican Senate — to give farmers emergency credit assistance. Reagan told Congress "someone must stand up to those who would say, 'here's the key to the Treasury — just take as many of those hard-earned tax dollars as you want' ".

For a President who four years ago handed those keys to Secretary of Defense Caspar Weinberger and has yet to ask for them back despite $200,000 million-a-year deficits, it was an interesting statement. Amid rhetoric that refers to that unprecedented deficit as some sort of natural phenomenon, it is perhaps easy to forget that it is in fact the direct result of the Reagan tax cuts and defence build-up. A glance at the accompanying graph, prepared by the non-partisan Congressional Budget Office, should be enough to set the record straight.

As for farm credit assistance, Reagan blamed the current troubles on "a generation of failed policies that drove down farm prices and drove up the costs of their land, seed and equipment", a statement that borders on a *non sequitur*, but which presumably reflects the President's instinctive dislike for anything that can be traced to Franklin Roosevelt's New Deal, rather than any precise statement of cause and effect. If the current credit crisis can be traced to anything, it is surely the expansionary policy begun under the Nixon Administration in which farmers were encouraged to plant "fencerow to fencerow". Farmers expanded; rural banks then pressed farmers to take full advantage of rising land values to borrow to the hilt. Farmers, straddled with high interest rates and coming out of several consecutive years of losses, cannot now get operating loans, and rural banks, having made questionable loans in the 1970s, are faced with insolvency; but the crisis cannot be blamed on a "generation" of government intervention — rather, the blame is with an ill-thought-out burst of *laissez-faire* enthusiasm under Nixon that Reagan is repeating under the rubric of a "market-oriented" policy. The lesson of the 1920s, when *laissez faire* reigned, was that overpopulation is a chronic, recurrent disease in agriculture. Then, as now, the salvation of US farmers was supposed to be exports. The lesson has not apparently been learned that the world does not want to be fed by the United States, except in dire emergency.

It is tempting to view the current farm crisis as the inevitable consequence of an economy undergoing "restructuring"; the end result, in this happy view, is a leaner, more efficient economy. That is the view of David Stockman, director of the Office of Management and Budget, at least, That view is reinforced unwittingly by those proponents of assistance who say the government must help to preserve the "way of life" of the family farmer. An uncritical look at statistics also suggests that small farms are inefficient and might well vanish. But this is a pitfall. It is necessary to understand that the statistics call anything a "farm" that has a annual output of agricultural product worth $1,000. That definition pulls in thousands of farms that are really not; some 70 per cent of all "farms" have sales of less than $40,000 per year, and these are about evenly divided between those owned by people who work elsewhere and farm mainly for amusement and those owned by older farmers who paid off their mortgages long ago. They are inefficient and produce a negligible amount of the nation's food. They are not the people in question.

Those who are calling for help now are the full-time family farmers, who, according to a 1981 Department of Agriculture study, are virtually as efficient as the very largest farms. (The study found that almost all economies of scale are attained by farms of about 300 acres, roughly the national average.) Expansion, and the inevitable elimination of some operators, occurs not because the smallest are inefficient, but because the smallest simply cannot produce enough income to support a family. Society does not gain from further expansion and concentration of production in fewer hands among this group. Yet that is the inevitable consequence of policies that foster overproduction, depressed prices and the substitution of capital for labour without any gains in overall cost efficiency.



There are really only two points which argue for continued government intervention. One is that society has a legitimate interest in preserving diversity, both geographical and economic, in the farm economy, so long as efficiency is not harmed. Second, again as the 1920s should have taught us, is that periodic booms and busts are harmful and inevitable in an unregulated market made up of producers with a high capital investment who sell what economists call perfectly competitive goods. Farmers are price takers; they must accept whatever the wholesaler offers. And even when those prices fail to meet costs, farmers have little choice but to continue producing (exacerbating the overproduction that depressed prices in the first place) in the hope of making good on their sizeable capital investment. General Motors can stop buying parts when demand falls; Sears can stock new items in its warehouses; but a farmer can do only one thing with a milking parlour.

The Reagan Administration is correct in saying that the current mess of contradictory farm programme has to go. A rational public policy aimed at intervening in the farm economy to level out the peaks and troughs need not cost a sum that approximates total net farm income (as it has in recent years). It certainly does not need programmes that encourage production.

First, instead of throwing up its arms and shouting "compete", the government could limit subsidies to the first increment of production per farm; the current system of "target prices", which operates at the margin, is an out-and-out production incentive that has no place in a rational solution. The second, more drastic, step is to be tougher in tying *all* federal assistance to a farmer's acceptance of rigid production limits. Farmers resist this, as a

matter of pride and to hold to their cherished image of themselves as rugged individualists. The New Deal programmes that have been inherited were attempts to plan the farm economy by doing everything but; it is time to drop the pretence and save a few billion dollars in the process.

Agricultural research, which is a metaphor for the sickness of overall farm policy, has also suffered from short-sightedness. Research programmes arise from the mists to appease some local constituency or deal with some emergency. Painfully absent has been a long-term commitment to solving fundamental problems. A genuine investment in basic research in plant molecular biology — which means considerably more than the still-token effort to establish a peer-reviewed grant mechanism for agricultural research — could pay off in lower input costs to farmers by cutting their dependence on energy-linked tillage, fertilization, irrigation and pest-control costs. That in turn could do much to reduce the expansionary pressures on farmers that are now answered by taking out more and more loans and overproducing.

Imbalanced budgets

Next week's British budget could be decisive for the future of British industry and innovation.

CAN governments do anything at all to revitalize an economy fallen on bad times? In a blend of disappointment and despair, this is likely to be the Britsh government's unspoken question about its revenue budget for the year beginning 1 April, due next week. For outwardly the government should now be basking in the rewards of six years of virtue. Learning from the mistakes of its predecessors since 1964, but also for doctrinal reasons, the present government has avoided, almost, direct intervention in the pattern of industry. Consistently it has been saying that governments can hope only to create the climate in which economic growth and thus prosperity will emerge. But eschewing direct intervention, governments must fall back largely on fiscal measures, whence the importance of next week's budget. The worry for the British and their government is that six years of almost consistent budget-management have so far produced only one tangible success — the inflation rate is a modest five per cent a year. Otherwise, unemployment continues to grow while the slow growth of the Gross National Product in the past few years consists largely of the expansion of service industries. Manufacturing industry (apart from oil) is still producing less than in 1979. And now interest rates are higher, and sterling lower, than ever before.

What has gone wrong? Why has so much effort been so poorly rewarded? From the outset, one of the government's key objectives has been to reduce the proportion of the national income spent by local and central governments, in the twin belief that the proportion has become too high and that, if the level of taxation were reduced, people would have more to spend in ways that would stimulate the economy. More recently, the same British government has been saying that direct taxation (on income) should be replaced by indirect taxation (on spending) because people could then choose investment rather than consumption.

Three things have caught the British government napping. First, public spending has remained high as a proportion of the total because of the growing social costs of unemployment and longevity, but also because spending on essential services such as education is so meagre that it cannot easily be cut. Second, when people have extra money because they pay less tax, they (or the financial institutions that hold their savings) are as likely to invest in industry elsewhere as in Britain. That is why there is something in the view of those economists who argue that restrains on capital outflows would bring immediate prosperity. (The snag is that the short-term would be very short.) Third, the government's actions have not been nearly as consistent as its rhetoric. Last month's sterling crisis seems to have been partly triggered by a belief that the government was allowing the money supply to grow in a way contrived not to show up in the accepted monetary statistic—but can it make sense to spend at least £10,000 million to replace Polaris nuclear submarines by Tridents at a time like this? (An Anglo-French accommodation on nuclear weapons would be cheaper and militarily prudent.)

Signs that the British government is beginning to lose its composure are, in the circumstances, inevitable but significant. With the sterling crisis, last month was replete with signs of fraying nerves. Even more remarkable was an attack delivered last Friday by Mr Nicholas Edwards, Secretary of State for Wales, on the financial institutions of the City of London. Mr Edwards accused them of "prejudice, ignorance and a striking lack of awareness" of what is happening in modern industry. He singled out an electrical and defence contractor, the General Electric Co PLC, for having accumulated large amounts of cash (in excess of £1,500 million) and for turning itself "into an investment bank" rather than investing in innovation. But what else can Mr Edwards expect, when his own government is prepared to offer people a rate of interest as much as ten per cent above the inflation rate, and when the rewards of innovation are unlikely to be as great?

That is the clue to the fiscal part of the solution to the problem of encouraging reinvestment in British enterprise. British interest rates are at present high not merely because they are tied to interest rates in the United States but because of the money mismanagement rumbled a month ago. The way to bring them down, and to cut the inflation rate, is further to reduce public spending. Tax cuts would work in the same direction but less quickly. The government's difficulty is that it has cut most things to the bone already, so that there is little scope for further manouevre. Sooner or later it will have to bite the defence bullet, and the sooner the better. Certainly there is little hope that productive investment can be stimulated by devices such as the ill-starred Business Expansion Scheme, which offers exemption from income tax for five-year investments in new business, and which seems chiefly to have stimulated property development. The moral, for those who like their fiscal policies to be clean and tidy (like the present government) are that a belief that the market knows best is incompatible with attempts to rig it. The moral for those who say that Britain is good at invention but poor at exploitation is that the explanation may lie in fiscal mismanagement. □

Polish normalcy?

Is the Polish government serious in saying it wants normal relations with the outside world?

THE account of Polish science by Vera Rich on page 123 says nothing of the circumstances in which the information was gathered. Ms Rich left London for Warsaw three weeks ago, on a visa provided by the Polish Embassy in London. The visa was due to expire at midnight on Tuesday, 26 February. Protesting in London that she planned to return on a charter flight only on the following day, 27 February, Ms Rich was told that there would be no difficulty if she left on the first flight that day.

On the morning of 27 February at Warsaw airport, the first flight was delayed for several hours, until 11 a.m. On exit, Ms Rich was told that her visa was not in order, she was stripped and searched, a number of documents and audio-tapes were confiscated and Ms Rich was bundled into the waiting aircraft. She returned to London in great distress, humiliated and shocked.

The Polish government is understandably ambivalent towards journalists, especially those from abroad. It rightly regards a reasonable trickle of such people as a proof that normalcy has returned, but it cannot apparently stomach, or even understand, journalists' inevitable curiosity about the events of the past four years. Not that Ms Rich's too-brief visit to Warsaw last month was in any way subversive — most of her meetings with officials were arranged under government auspices anyway. The Polish Embassy in London has not yet replied to a request for an explanation of why Ms Rich was bundled out of Warsaw in such an outrageous fashion. This note is meant to jog the embassy's memory. The explanation, if forthcoming, will of course be published. □

Nuclear winter

Pentagon says yes, it may happen, but "so what?"

IN its first official statement on "nuclear winter", the Department of Defense (DoD) has acknowledged the possibility of severe climatic change following a major nuclear war. But the DoD statement, which comes in the form of a report ordered by Congress*, asserts that the only policy implication is to affirm current US strategic doctrine on deterrence, arms control and Star Wars missile defence.

The DoD report echoes an earlier study issued by the National Academy of Sciences last December in its criticism of the large uncertainties in published estimates of the climatic effects of nuclear war. "At this time, for a postulated nuclear attack and for a specific point on the earth", the DoD report says, "we cannot predict quantitatively the materials that may be injected into the atmosphere, or how they will react there . . . . we do not expect that reliable results will be rapidly forthcoming."

DoD specifically criticizes uncertainties in the results of the study by Turco *et al.* (*Science* **222**, 1283; 1983). It says the estimate of the amount of smoke that would be produced in fires ignited by nuclear blasts was the result of combining a large number of poorly-known variables; "in actuality, the same yield weapon could produce vastly different amounts of smoke over different target areas and under different meterological conditions", DoD says.

A preliminary DoD assessment of 3,500 "hypothetical" non-urban target locations concludes that smoke production would be 30 times less than the Turco *et al.* assumption for July and almost 300 times less for January**. The DoD report also suggests that the results may have improperly shifted the focus from dust to smoke; if smoke production has been overestimated, and if one assumes a war scenario in which, for example, ground-burst attacks on missile silos predominate, producing a lot of dust, the effects of dust may outweigh those of smoke.

The DoD report also claims that initial results of three-dimensional models constructed by DoD and National Center for Atmospheric Research scientists show that scavenging of smoke and dust from the atmosphere may be "substantial".

DoD is spending $1.5 million on nuclear winter research this year and has requested $2.5 million for next year. An additional $2 million per year is coming from the Department of Energy.

But whatever the outcome of these studies, DoD is already suggesting that they will have no effect on US strategic policy. DoD claims credit in the report for having already adopted policies designed to limit both the extent of nuclear war and its atmospheric consequences. It claims that US policy is to avoid urban population centres in its targeting plans, a policy that would also limit bomb-ignited fires.

Significantly, DoD also uses the opportunity to push President Reagan's Star Wars plans, noting that even "single-layer defense" — perhaps a reference to a terminal site defence, the one star wars component that may be possible with existing technology — "may provide a greater mitigating effect on atmospheric consequences than could result from any level of reduction likely to be accepted by the USSR in the near term".

The report dismisses the argument that the Soviets would respond to a US anti-missile defence simply by increasing their offensive forces, claiming that since the signing of the anti-ballistic missile treaty in 1972, the Soviets have increased their forces fourfold. The report does not mention the US decision to arm its intercontinental ballistic missiles with multiple warheads during that period.

The clincher, as far as the Pentagon is concerned, is that even if the United States becomes convinced of the reality of the nuclear winter, "we cannot be confident that the Soviets would expect such effects to occur as a result of all possible Soviet attacks".

The DoD report claims that the Soviets have performed no independent calculation on climatic effects of nuclear war and in fact "show no evidence of regarding the whole matter as anything more than an opportunity for propaganda".

Stephen Budiansky

* *The Potential Effects of Nuclear War on the Climate*, A report to the United States Congress, Secretary of Defense, March 1985

** *Smoke Production from Multiple Nuclear Explosions in Wildlands*, R D Small and B W Bush, Pacific Sierra Research Corp in the press

ASATs

US Air Force slows the pace

Washington

ALTHOUGH the congressional ban on the further testing of the US anti-satellite weapon (ASAT) expired last week, new tests have been postponed at least until June. Technical problems are partly responsible for the delay, but diplomatic concern about the Geneva arms control talks, due to open next week, may also have been influential.

The Air Force officially refuses to discuss the technical problems or the testing schedule of ASAT. But administration officials last week quietly spread the word about the technical delays. And according to *Commerce Business Daily* of 21 December 1984, the Air Force has asked for bids on a contract for the redesign of the ASAT's "maneuvering propulsion package", a group of 57 miniature solid-fuel rockets that steer the 12-inch ASAT projectile into its target.

The contract announcement mentions contamination from motor exhaust as one problem to be corrected. According to John Pike, a space-weapons expert with the Federation of American Scientists, contamination of infrared sensors has been a "canonical problem" with this type of weapon, going back to prototypes developed for ballistic-missile defence two decades ago. Infrared sensors are used to pick up the heat radiated by the target satellite and so guide the projectile, technically called the "miniature homing vehicle". The projectile carries no explosives, but disables the satellite simply by colliding with it.

So far, the Air Force has tested ASAT only by shooting at a point in space. Live targets have not yet been used. (According to some reports, for the "point in space" test last November, ASAT's infrared sensors were locked on a star or the planet Jupiter.)

Although the congressional ban on testing against live targets has now expired, the administration would still be required to send a lengthy certification to Congress before such a test could proceed. When the time comes, ASAT opponents say, the administration will have a difficult time completing that procedure with a straight face. Certification will require a finding that the test is necessary to avert "irrevocable harm" to national security, that the United States is trying to negotiate limitations of ASATs, that the test would not irreversibly harm prospects for negotiations and that it is consistent with the anti-ballistic missile treaty.

Representative George Brown (Democrat, California), one of the sponsors of the congressional restraints on ASAT testing and one of 102 congressmen who signed a letter to President Reagan last week urging a continuation of the moratorium, has suggested that the technical problems may be a convenient refuge for an administration that wants to keep up a tough image while not directly jeopardizing the Geneva talks. And Pike said, "I assume that, if they thought the safety of the republic was in jeopardy, they'd be working overtime [to correct the problems] but they've adopted a leisurely schedule".

A middle course that the Air Force may be contemplating is a second test against a point in space in June or July, which would not require the certification.

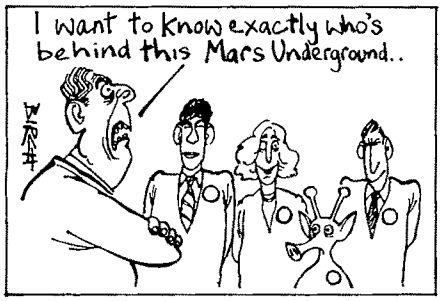
Stephen Budiansky

US space science

Manned flight to Mars?

Washington

ATHOUGH the National Aeronautics and Space Administration (NASA) is officially keeping its distance, a dedicated group of space scientists within and without the agency is busily hatching plots for a manned space flight to Mars. The so-called "Mars Underground" has held several conferences to discuss ideas for the mission, which, members of this loose network of enthusiasts say, could be done for as little as $17,000 million, and largely with



technology already available or being developed for NASA's space station.

The National Space Institute, a group of space buffs that sponsored two of the conferences, has even taken to distributing "Mars Underground" lapel buttons to be "quickly flashed" at first sight of the NASA administrator, senators and congressmen.

The rise of the Mars underground has highlighted a growing rift between those who favour continued unmanned exploration of the planets — mainly planetary scientists concerned about getting the most data per dollar — and those pressing for a project that would rekindle the "thrill of exploration" that was the main dividend of the Apollo flights to the Moon. Carl Sagan has recently been trying to bridge the gap, conceding that a manned mission is not justifiable on a scientific basis alone, but urging it nonetheless in the form of a joint US-Soviet venture "on behalf of the entire human species".

NASA's current plans for Mars are limited to the unmanned Mars Geoscience/Climatology Orbiter, scheduled for launch in 1990. Since the 1975 Viking 1 and 2 Mars landers, which raised many questions about the Martian surface, planetary scientists have been talking about follow-up missions involving a surface rover, a remotely-piloted vehicle and a return of a surface sample to the Earth.

To the extent that NASA is giving official attention to a manned Mars flight, it is in the context of advanced planning for the space station. Jesco von Puttkamer, NASA's programme manager for long-range studies — an office sometimes derisively described as NASA's division of daydreaming — says that a Mars flight is just one of five general categories of projects that an advanced space station could make possible; the others are low-earth-orbit instruments, commercial development of geosynchronous orbit, Solar System exploration with unmanned probes and establishment of a permanent lunar base. A space station would be a necessary jumping-off point for a Mars mission because of the sheer mass involved. Puttkamer says that a bare minimum weight for a Mars craft would be 2 million pounds; the huge Saturn V rocket built to launch Apollo to the Moon had a capacity of only 250,000 pounds. The space shuttle carries 65,000 pounds. Thus, assembly in Earth orbit is a necessity.

Puttkamer estimates the cost of the first Mars flight at $50,000-65,000 million; even that figure assumes a larger shuttle and an advanced space station would already be available. And because the Earth and Mars are in a favourable orientation for a transit only every two years, a one-shot flight makes little sense. Nobody knows how much a continuing programme of Mars flights, including establishment of a Mars base, would eventually cost.

The more enthusiastic enthusiasts, such as the Planetary Society, put the costs much lower ($17,000 million) and argue that new technology (such as aerobraking — using the atmospheres of Mars and the Earth to slow the craft down, instead of retrorockets) could cut fuel demand — and thus weight — considerably.

Some have also tried to raise the familiar Red Menace (which was good enough to get a man to the Moon) as a reason for pressing ahead. But according to a Congressional Research Service study by Marcia Smith, the Soviet capability argues against a serious effort on their part to put a man on Mars. The Soviets are apparently planning to send an unmanned probe to Mars's moon Phobos in 1986. But despite continued interest in Mars and a growing expertise in long-duration space flight, Soviet space hardware continues to be plagued with low reliability. Only one of the eight Soviet Mars probes has been an unequivocal success; the rest have missed the planet, crashed or failed to return data. And the Soviets have yet to try putting a man on the Moon. **Stephen Budiansky**

Biotechnology

Agrigenetics forced to merge

Washington

THE acquisition earlier this year of Agrigenetics by Lubrizol Corporation may herald a new trend in the biotechnology industry. Things have come a long way since the heady days of summer 1983, when it seemed that any company offering expertise in the new technology could do no wrong in the eyes of investors. By early 1984, public offerings were getting a much cooler reception, making difficulties for some companies seeking development capital. Agrigenetics, based in Boulder, Colorado, had seemed one of the industry's brightest hopes, having an impressive array of research expertise. But a combination of high interest rates and a depressed agricultural products market, together with the decline of the high-technology securities market, eventually forced it to seek a merger with a much larger company.

Lubrizol is saying very little about its business plans, but does not intend to alter the direction of Agrigenetics' research, which may even be augmented. But there must also be some doubt whether Lubrizol, as sole owner, will want to renew the limited partnership deal it struck with Oppenheimers of New York in 1981 and which expires in 1986. That programme, which supports research worth $11 million a year in 24 universities across the country, would be missed by many.

Analysts are cautious about generalizing too much from the Agrigenetics experience, since its business strategy was unusual from the start. Under chairman and founder, David Padwa, the company acquired seed companies to form a distribution network at the same time as expanding its research. Reliance on the agricultural products market may have increased its vulnerability with US agriculture in the doldrums. The research arm was spending $19 million a year principally on developing new crop varieties by applying new techniques such as the microinjection of DNA into plant cells, the use of Ti plasmids and the exploitation of transposable elements. By having a distribution network already in place, the company hoped be able to steal a march on competitors when products of the new technology eventually reached the market.

Things began to go wrong in November 1983, when the company decided to abandon a public share offering because of the deteriorating market. Looking instead at merger possibilities, Agrigenetics found Lubrizol, a manufacturer of special oils and oil additives, a likely mate. The company already owns 15 per cent of Genentech Inc., regarded by many as the Rolls-Royce of the biotechnology industry, and was seeking ways of using genetic engineering technology to produce improved vegetable oils.

Outstanding Agrigenetics shares were exchanged for 2.1 million Lubrizol shares worth $48 million, and in addition Lubrizol took on Agrigenetics' debt of $60 million. In the circumstances, Agrigenetics shareholders could have done much worse. Padwa resigned as chairman last month, but denies differences of opinion with the new owners over strategy, and may continue to serve occasionally as a consultant. **Tim Beardsley**

Poland

New directions for science

NEXT October, a grand "Congress of Polish Science" will be convened in Warsaw. The initiative for the Congress came from the government and party authorities, not from the scientists, most of whom seem to know little, so far, of the purpose of the proposed gathering. It seems, however, to be intended as a working meeting, not as a demonstration to the outside world that all is well with Polish science. For, according to Dr Ignacy Malecki, a leading member of the Polish Academy of Sciences, no foreign visitors will be invited. (Until his recent retirement, Dr Malecki regularly represented Polish science at the quasi-diplomatic level of ICSU and similar meetings.)

This does not mean that Polish science is going isolationist but rather that the congress, which, Dr Malecki hinted, will include a major session on technology and innovation, represents a determined effort to put science on a new path. For the present system of research support, based on a complex hierarcy of "problems" from the "governmental" down to the "branch" level, is in disarray and is expected to be phased out at the end of this year, when the current five-year plan comes to an end. Last December, the former Ministry of Science, Higher Education and Technology lost its responsibility for technological research and innovation, which was handed over to a new State Committee for Technical Progress that will be specially empowered to direct research funding to those areas likely to provide a short-term economic pay-off.

Even more significantly, science funding for the next five-year plan will give priority to fields such as computer science, electronics and biotechnology that were judged to be of particular contemporary significance by the recent Comecon summit. The support will be generous by Polish standards; in the case of biotechnology a sum of 15,000 million zloty (£100 million) has been mentioned, although some of this, at least, must be made available in hard currency, if even these leading areas are not to suffer from the endemic problem of contemporary Polish science — a lack of funds to purchase from the West journals, reagents and spare parts and replacements for ageing equipment.

These announcements have been received by the Polish scientific community with mixed feelings. The old system of funding by "problems" was universally unpopular. It caused considerable administrative difficulties whenever an institute wanted to buy some standard equipment, say a microscope, to be used by several teams working on different "problems", and ate into the time of the unfortunate members and employees of the Academy of Science who had to coordinate the efforts of scientists working on the same "problems" in institutes scattered all over Poland. The large sector of "branch" research institutes, belonging neither to the academy nor the universities, is in considerable confusion, and few would question, in principle, the need for new legislation to cover that sector. Even the increased emphasis on practical applications is viewed by many as an unfortunate necessity of Poland's continuing economic crisis.

What the scientists find less acceptable, however, is that decision-making on priority investment in Polish science has, effectively, been transferred from Warsaw to Moscow. They fear, too, that upgrading research with practical applications will be used to justify cutbacks in other sectors, so as to cover a purge of political undesirables, terminating their research contracts on the grounds that no more funds are available. A painful example to scientists and scholars of every discipline is the fate of the Institute of Nuclear Research (IBJ) which in the winter of 1982–83 was dissolved and refounded as three institutes to weed out pro-Solidarity personnel without formally contravening the labour laws. If this could happen to IBJ, they argue, at a time when the government had just relaunched its nuclear energy programme, what are the prospects for an astrophysicist, a logician or a sociologist who falls foul of the system?

Not surprisingly, the trauma of martial law still haunts the scientific community. Formally, of course, all was forgiven and forgotten under the amnesties of 1983 and 1984. Interned and imprisoned scientists have been reinstated and the situation at IBJ officially explained as voluntary redundancies, premature retirements and the like. In fact, some of those reinstated under the amnesties have since been dismissed or eased out of their jobs (anecdotal evidence suggests that this process has been stepped up since the beginning of this year), while some formally convicted of offences against the martial law regulations have not been able to return to professional employment.

There seems to have been little consistency; of the two physicists on trial for anti-state activities when the 1984 amnesty put an end to the case, Dr Henryk Wujec has been allowed to return to his old place of employment, the Standards Office (through transferred to a different department), while Dr Zbigniew Romaszewski is unable to find a scientific post at all. A mathematics student convicted of distributing leaflets under martial law has found that, after graduation, he cannot obtain a job, whereas a class-mate similarly convicted has found no difficulty.

In some cases, exemplary punishment seems to have been inflicted almost at random: Dr Zbigniew Grabowski, a physical chemist, for example, had his election to the Academy of Sciences vetoed by the government, although his only "offence", like that of hundreds of his colleagues, was signing a petition against the imposition of martial law.

A particularly umpleasant penalty, which has revived the fears of the whole scientific community, was that inflicted on Dr Zofia Kuratowska, who, during martial law, had headed the medical service for the internment camps. Dr Kuratowska is employed by the Advanced Studies Centre of the Polish Academy of Medicine, and runs advanced training and updating courses for doctors and medical graduates from all over Poland.

In 1983, just as martial law was coming to an end, she says, she was informed by the security police that they intended to put an end to her professional career by closing down her clinic at the Barska Street a hospital in Warsaw. In spite of petitions from doctors, patients, nurses at the general public and last-minute appeal to the Council of State, the clinic was closed on 31 December 1984 (ostensibly because the wards were needed for other purposes), leaving 500 haematological and geriatric patients without proper medical cover, and interrupting the postgraduate courses.

Dr Kuratowska has found temporary accommodation for the courses (at least until the end of the year) at the partly-built Oncological Centre Ursynow, on the edge of Warsaw. She has no in-patient facilities there, however, and, she reports, five patients have already died as a result of the closure.

Such incidents, sporadic as they are, are sufficient to make the Polish scientific community apprehensive for its future. Already the autonomy of the universities, won in the Solidarity period, is under attack and the proposed new law on the Academy of Sciences, which was still being drafted when martial law intervened, has been quietly shelved.

A major innovation of that law would have been to make the academic secretary of the academy responsible to his fellow members and not, as at present, to the prime minister. How significant such a change would have been may be illustrated by the Gradbowski case: not only was his election to the academy vetoed, but the very fact of the election was stricken from the academy records.

To an increasing number of Polish scholars, the prospect of a purge is now only a matter of "when" and "how severe". At present, scientists and academics unable to find professional work can try to continue their theoretical research privately. An unofficial Social Committee for Learning administers a few bursaries for such scholars, at parity with the official pay scales for academy researchers (about two-thirds of the national average salary). A scheme that can help a couple of dozen distressed scholars however, is clearly not equipped to cope with the victims of a major purge.

Vera Rich

French science

Good times are here again

THE French socialist party, which forms the present government in France, took a knock at the polls last weekend when right-wing parties scored 58 per cent of the vote in local elections. Of course, the French economy took its dive later than others in the West, and the electorate blames it on the new government. They may be right. But President Mitterrand's administration does have at least one feather in its cap — science — and it was seen to be waving it vigorously last week before the elections.

According to a communiqué from the council of ministers last week, French research and development spending has leapt more than a fifth in real terms since 1980, from 1.85 per cent of gross national product in 1980 to 2.25 per cent this year. Basic science may not have done so well (against a rising dollar) but "France has thus begun to regain its position among its principal partners and foreign competitors", the communiqué claimed. The benefits would come later, the investment "furnishing a solid base or the second step in the politics of industrial renovation".

The trouble is that the first step has been restructuring and unemployment. The electorate, unfortunately for the government, observes decline now more clearly than growth later. Nevertheless, the government has again pinned its colours to scientific growth at an important moment, when the "law for science", the creation of the first socialist science minister, Jean-Pierre Chevènement, nears the end of its term.

To replace it, Hubert Curien, the present science minister, promises a new law to be presented to the National Assembly in the spring, while the council of ministers is announcing that "research and development are, in effect, the keys to the modernization of our country". Auspicious times for French science appear to be here again.

Yet there are problems. Curien has been making an assessment of the successes and failures of science policy over the past three years, and describes it as "incontestably positive". But industry has been slow to invest in research; the new labour rights of researchers (which are supposed to increase mobility) have only just come into force, and many basic research laboratories are suffering from under-equipment and too few hard dollars for materials and apparatus. French computing power is also way behind the competitors, despite a decision last year to allow laboratories — after ten years' moratorium — to buy foreign machines. Much cash was also wasted on ill-conceived national technology programmes.

The necessary structural reforms have now been accomplished, says Curien; the great revolution has been the acceptance by a largely left-wing university system of the need to cooperate with industry and by industry of universities as partners. The stage is set for real development, Curien would claim. "The decline of Europe in relation to the United States and Japan is not written in history", he wrote last week in the newspaper *Le Monde*. And France must develop with Europe: "Is science not one of the best cements for a still fragile but indispensable European cooperation?", he asks.

In France, a commission has been set up to study the proposed law for science. But already the council of ministers has promised scientists several benefits:

- An increase in cash available for laboratory equipment.
- A long-term policy for training and employment in research and development.
- The establishment of research more widely in industry.
- Continuance of the "great programmes of technological development" such as space, aeronautics, energy and the oceans.
- At the same time, reinforcement of the assessment of these programmes and other action so that corrections can be applied when things go wrong.
- Improvement of tax and other financial incentives to industry and to research.
- Continuance of the "strong priority" given to research and development.

Robert Walgate

Italian biotechnology

Planning to catch up rivals

Rome

A NEW centre for biotechnology research and development is being founded at Pomezia, 45 km south of Rome, by Menarini SaS, the Florence-based pharmaceutical company. Dr Alberto Aleotti, president of Menarini, says the company is investing 28,000 million lire in the new biotechnology centre.

The first goal of the centre will be the manufacture of TPA (tissue plasminogen activator) and its derivatives for human pharmaceutical purposes, using recombinant DNA technology developed by Creative Biomolecules, a California corporation founded in 1982 by the 36-year-old Italian, Roberto Crea, and Charles M. Cohen.

The agreement between Menarini and Creative Biomolecules is aimed at the production of an economic drug for the international market. The Pomezia team will include researchers from Italy, the United States and Britain. The first TPA for chemical and biological evaluation is expected in the summer. Crea, acknowledging that his project is not the first in the field — Genetech first cloned TRA in 1982 — says its objectives are superior.

Inaugurating the Menarini centre (and the toxicology research facility on the same site), Italian minister of research Luigi Granelli pointed to several encouraging signs of development in Italian biotechnology by way of international cooperation. The international genetic engineering centre of the United Nations Industrial Development Organization, to be based at Trieste, is one of these. But Granelli added that developments in this field in Europe as a whole are still not a sufficient counter to developments in Japan and the United States, perhaps because governments see their interests as stronger than supra national ventures.

Italian biotechnology is in part held back by the lack of venture capital, with the result that most of the companies involved are either well established in some related field such as pharmaceuticals or are public enterprises. The companies with declared interests include Farmitalia-Carlo Erba (Montedison group, now under the umbrella of Erbamont), Lepetit and Serono. Sorin Biomedica (wholly owned by Fiat), Farmitalia and various companies of the ENI group have declared an interest in protein chemistry; Sclavo, an ENI company, plans to produce beta-interferon. Biotechnology in agriculture is quite advanced at ENEA, the public corporation for nuclear and alternative sources of energy, and Assoreni (ENI group) has interests in agricultural biotechnology.

University researchers have been drawn into biotechnology in various ways. Thus the multinational Roche facility at Milan has an agreement with the Pharmacology Institute of the University of Milan, headed by Professor Rodolfo Paoletti, for applications to molecular neurobiology. The Italian Society for Biotechnology (SIB) has signed a convention with IASM, the public sponsor of development in the south of Italy, and with FINAM, a financial holding for agriculture, to set up a centre for biotechnology transfer in the south.

The first objective of the centre will be to evaluate the degree of innovation in small and medium-size industries so that by the end of 1985 agricultural experiments on a pilot-plant scale may be started.

Projects sponsored by the Consiglio Nazionale delle Ricerche (CNR) have modest achievements to report. While the CNR genetic engineering programme, launched in 1982 with 20,000 million lire has so far recruited only a few participants among Italian industries, a 1980 project (due to finish this year) on fine chemicals under the direction of Professor Luciano Caglioti has worked with 500 different entities. So far, the project has published 1,800 scientific papers and produced 150 patents, some of which have already gone into production.

Paola de Paoli

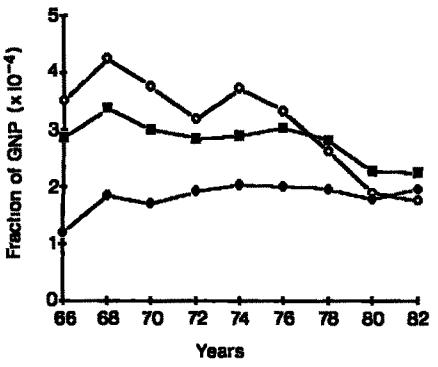
CERN

Reduced budget in prospect

Geneva

BRITISH officials are beginning to sound out the 13 member states of CERN, the European Organisation for Nuclear Research near Geneva, to see if they would countenance a substantial reduction in the CERN budget (which amounts to some £250 million a year) while a fourteenth potential member, Portugal, has applied to join. Could Portugal pay for the reduction Britain requires? Certainly not, but the eagerness of Portugal and the dog-in-the-manger attitude of Britain make a sharp contrast seen from CERN.

Even Britain's backing of salary increases at CERN (*Nature* 7 February, p.423) could be a ploy in the long run of reducing salaries; negotiations begin this year for a major two-year review of the CERN salary structure. Britain, it is said, did not wish to begin on the wrong foot from the start of these negotiations (which Britain will chair) by backing a sudden and unprepared French move to cut pay.



The decline of Britain's spending on high-energy physics (open diamonds) as a fraction of gross national product, compared with that of West Germany, France, Italy and the United Kingdom jointly (full squares) and Belgium, the Netherlands, Sweden and Switzerland (full diamonds). Salaries are included.

By 1982, UK spending was already below that of the latter "medium size" members of CERN.

Thus Britain's head-counting looks ominous — particularly in the light of a committee chaired by molecular biologist Sir John Kendrew which will report soon on the value of Britain remaining a member of CERN. Sir John may praise the performance of CERN — it is, after all, at an all-time high in its history and even leading the world — but he may also criticize costs

Britain's real spending on high-energy physics has been declining rapidly (see Figure), but European investment remains a third above that of the United States when measured as a fraction of gross national product. CERN argues that this reflects the superior quality of CERN accelerators, and that without this quality Europe would not be in such a commanding position. Fermilab in the United States is three years behind CERN in creating the antiproton-proton collisions that at CERN created the W and Z particles and now hint at supersymmetry. But British accountants may find the benefits imponderable.

CERN, however, says that their next big accelerator (the large electron-positron collider, LEP) is being built on constant budgets, so that cuts are already being made throughout CERN to pay for LEP's construction. The director-general, Professor Herwig Schopper, is cutting staff, and accepts a Thatcherite hypothesis that every organization has some fat to lose. "But we're now cutting to the bone", says research director Ian Butterworth.

The bone is research. Over the past five years CERN has run double the number of research hours of its most direct competitor, Fermilab, says CERN technical director Giorgio Brianti. "We run a good deal — 7,000 hours a year, more than most nuclear power stations." But research after all is what investment in CERN is for.

Yet to cut running time, and hence electricity bills, might be the only real cut that CERN could now make. Already 70 per cent of the capital spending on LEP is committed. And salaries (half the CERN bill), while high by national standards, are low by international ones.

If running time were cut, physics would slow down, and competition from the United States would be more threatening.

Britain's possible complete departure from CERN is greeted with horror and disbelief in Geneva. CERN claims to have no contingency plans for the loss of Britain's 17 per cent contribution, or its physicists, though there would be a year or two to act (the constitution requires withdrawal on the January after the January following announcement of withdrawal).

Carlo Rubbia, the Italian discoverer of the W and Z particles, says Britain should go if it wants to, and not hang about like the character in Verdi's opera *Aida* who sings "I'm going, I'm going" for five acts but never leaves. Bob Brown, a British physicist in one of the four giant experiments to be mounted on LEP, says Britain's leaving "is too awful to contemplate". His experiment, OPAL, in which Britain has a major contribution (the provision of a lead-glass scintillator) "would collapse" if Britain left. Another LEP experiment, DELPHI, might be threatened.

Others say Britain just could not spend effectively the £50 million that the Science and Engineering Research Council would be left with if Britain left CERN; and that the research students presently staying in Britain to do high-energy physics (80 per cent of them with first class degrees) would simply go to the United States, weakening British science. In the longer run, non-members do participate in CERN experiments, but they make triple the normal contribution to apparatus, and Britain's problem would not thereby be solved.

"It would be a disaster" if Britain pulled out, said Schopper last week. "Would we throw out the British physicists? I cannot see that. But what would the other states say?". There would, in the end, be a limit to how long they would be willing to bail out a defaulter. **Robert Walgate**

No home yet for radiation source

THE European Synchrotron Source (ESRS), the 5-GeV light source that France and West Germany agreed should be built in France (in exchange for a supersonic wind tunnel to be built in Germany), is up for discussion yet again.

Now it is the turn of the Levaux Commission, the supposedly intergovernmental body set up by the European Science Foundation with the original aim of deciding the location of ESRS. France and West Germany effectively spurned the commission and decided among themselves (with British assistance) to put ESRS in France, hoping that the smaller states on the commission would agree.

But they did not; the situation was further complicated when the French government switched choices from Strasbourg (close to the German border) to Grenoble (in the French Alps), probably for electoral reasons. West German officials had always favoured Strasbourg, and were severely put out (though philosophical) when the French government changed its position.

Thus, when the Levaux Commission meets this week, French representatives will be all but isolated. France needs cash support to establish ESRS, and it is conceivable that strong pressure will be put on France to change its mind yet again. The regional elections feared by the French government, which could have displaced Louis Mermaz, the socialist president of the National Assembly, from his seat on the Grenoble regional assembly and perhaps precipitated a national election, are taking place last Sunday and next, so the internal political pressure will soon have changed. Not a stone has yet been turned for ESRS.

So could ESRS go to Strasbourg after all? Not if Denmark or Italy have their way, as Denmark still champions Risø and Italy Trieste. Scientific site visits to both places have taken place since France proclaimed the site would be Grenoble, and Italy continues to contest very loudly on behalf of Trieste. Last week, however, Italian physicists meeting in Trieste were talking of a compromise — the building of a 1.5 GeV synchrotron radiation source to "complement" the X-radiation from the 5-GeV ESRS in the lower wavelengths (soft X-rays and ultraviolet). Robert Walgate

Imunovir defended

SIR — In a letter published on 31 January[1], Dimitri Viza makes a number of assertions about Imunovir (inosine pranobex) and the "aggressive publicity campaign" which he suggests has been mounted to promote it. M. Visa's intention seems to be to criticize not just the product but also the journals and newspapers (specifically *Nature*, the *Sunday Times* and the *Financial Times*) which published articles on Imunovir (which is spelt with one m). In doing so he has made some errors, which I would like to correct.

First, M. Viza states that ". . . this drug is already better known from its promotion than its results". More than 300 papers have appeared on Imunovir, including many in journals such as *Lancet*, *International Journal of Immunopharmacology* and *Antimicrobial Agents and Chemotherapy*. As an example of this "promotion", M. Viza states ". . . US drugstore bookstalls hyped the drug with a paperback which claimed a cure for herpes . . . Although the author did not support this claims with any scientific publications, the book claimed him to be 'an authority on herpes'". The book in question, *Herpes: Cause and Control*[2], is an independent text on herpes by Dr William H. Wickett, former director of Student Health Services at California State University, Fullerton. The book discusses a wide range of preparations used to treat herpes, including acyclovir, idoxuridine and Imunovir. However, the latter is at all times referred to as "the test drug": not once is any generic or brand name used, and Dr Wickett states that this is at the request of the manufacturers. It is difficult to see how a book can "hype" a drug without once referring to it by name. Moreover, the book does not claim a cure for herpes. I quote Dr Wickett: "We found that those patients taking the test drug were cured of their Herpes bout in nearly 60% of the cases at the end of one week. Those taking the placebo were cured, by the same criteria, in about 14% of cases." Assuming that M. Viza is a doctor, he should know that herpes simplex is a recurrent disease and that curing a "bout" is not at all the same thing as curing the disease itself.

Neither the US discoverers of Imunovir, Newport Pharmaceuticals, nor Edwin Burgess Limited which distributes the product in the United Kingdom, has ever claimed that it can cure herpes. To quote the *Financial Times* article[3], also criticized by M. Viza, "[The company] is quick to point out, however, that the product is not a cure for viruses like herpes, only a moderator of the disease".

This brings me to another assertion, that if Imunovir is effective in herpes, the number of patients in France, Germany, Italy and Spain, where the drug has been available for some years, would have decreased. Imunovir certainly appears to reduce the severity[4-8] and frequency[9,10] of recurrent attacks of herpes simplex, but as it is not held up to be a cure, the number of patients with herpes can hardly be expected to decrease. In addition, the number of cases is probably related more to increasingly relaxed sexual attitudes than to available therapy.

To move on to the criticism of the article in the *Sunday Times*[11], it seems to be the headline that has caused offence: "Pill for AIDS — but it may come too late". Headlines are written not by authors of articles, but by sub-editors. In fact the *Sunday Times* article referred to work in "pre-AIDS" patients in New York, the results of which were presented to an audience of genitourinary specialists in London and are at present awaiting publication. The work was sufficiently encouraging for similar trials to be set up both in London and the United States to see whether the findings can be confirmed. These are now under way. The statement in the *Financial Times*[3] — "Imunovir may be able to prevent AIDS" — also refers to the New York study. M. Viza takes objection to another statement from that newspaper which suggests that Imunovir shows promising potential in a variety of diseases. I would like to draw his attention to the words of the jury which awarded Imunovir the "Prix Galien" in 1982, an award established in France to recognize major therapeutic innovation. (Other products honoured by the award include rifampicin in 1970 and cimetidine in 1979.) The distinguished professional jury stated that Imunovir "has transformed the basis of immune therapy and opens new hopes in the field of medicine as important and diverse as viral diseases, autoimmune disease, allergy, parasitology and cancer".

M. Viza is right when he says that responsible lay publications should not give rise to false hopes about potential "breakthroughs", and I believe that we would all be glad to see an end to sensational headlines which bear little relationship to the article, and to the "wonder drug" type of story in general. I dispute the idea that the articles to which M. Viza refers, including the one in *Nature*[12], are anything but well-balanced, responsible pieces of reporting based on the scientific evidence available.

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1 Viza, D *Nature* 313, 344 (1985)
2 Wickett, W H Jr *Herpes Cause and Control* (Pinnacle, New York, 1982)
3 Rapoport, C. *Financial Times*, 10 October, p 7 (1984)
4 Corey, L *et al Clin Res* 27, 41A (1979)
5 Wickett, W H *et al 76th Annual Meeting Am Soc. Microbiol*, New Jersey 1976
6 Bradshaw, L J *et al 4th Int Cong Immun*, Paris 1980 (Abstr)
7 Lassus, A *et al 1st Sexually Transmitted Diseases World Congress*, Puerto Rico, November 1981
8 Bunta, S *13th Interscience Conference Antimicrob Ag Chemother*, Washington, September 1973
9 Chang, W-T *et al 13th Interscience Conference Antimicrob Ag Chemother*, Washington, September 1973
10 Galli, M *Lancet* ii, 331, 1982
11 Deer, B *Sunday Times* 14 October, p 9 (1984)
12 Wenz, C *Nature* 311, 404 (1984)

Status of IVF embryos

SIR — An unfertilized egg is both human and alive, being a detached bit of its mother's body; but it cannot develop further without something positive being done to it from outside, namely fertilization. Otherwise it must inevitably die and, since nobody would claim that it therefore has a right to be fertilized, some would argue that if the "right to life" depends upon being human and alive, fertilization should make no real difference in this respect. But there is of course a fundamental difference, because when the egg fuses with the sperm a *new* living human organism comes into being, biologically as distinct from both of its parents as it will ever be, and none the less so for the possibility that it may still be able to give rise to twins. This newly created organism has within itself the potential to complete its development as a human person, which it will normally do unless it dies of its own accord, or is killed by a procured abortion. Whether or not at such an early stage it is to be regarded as a "human being", or only potentially one, it is hard to see what difference this should make to its rights to be allowed to realize its full potential without being deliberately destroyed.

There is however an important difference between the potential for futher development possessed by an egg fertilized normally inside its mother, and an embryo produced artificially by *in vitro* fertilization (IVF) outside her body. This is that whereas the embryo *in utero* has of itself the full potential to complete its development without any further intervention from outside, that is not so with IVF, when it requires another positive act in putting it back into a woman's uterus.

So far as completing its development is concerned, therefore, the IVF embryo can be compared with an unfertilized egg, since neither can do this without some positive intervention from outside. It is this, rather than the small size and lack of any visible signs of humanity, that may justify the use of IVF embryos for experimental work expected to result in death, which would anyway be unavoidable whether or not the experiments had been performed. The ethical problems that arise when embryos *in utero* are to be intentionally aborted are certainly quite different from those involved with experimentation *in vitro*, since even from the earliest stages they would otherwise have had a chance of completing their normal development.

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Unborn Children (Protection) Bill

from H. John Evans and Anne McLaren

If the bill recently presented in the British Parliament were to become law, many promising avenues of research would be blocked.

MUCH to the dismay of most informed scientists, infertile couples and parents at risk of producing children with severe inherited genetic defects, Mr Enoch Powell's private member's bill effectively to ban studies on human embryos created by *in vitro* fertilization (IVF) won a majority when given its second reading in the British House of Commons, on 22 February.

The bill seeks to "render it unlawful for the human embryo created by *in vitro* fertilization to be used as the subject of an experiment or indeed, in any other purpose except to enable a woman to bear a child" (*Hansard*, 15 February, 1985). The bill does not seek to interfere with "procedures that are in use at present for enabling women who would not otherwise be able to do so, to bear children", but will, despite Mr Powell's comments to the contrary, effectively block any attempt to improve these, at present, rather inefficient procedures. The present success rate for human embryo transfer is of the order of 10 per cent. There is, however, no obvious reason why medical science should not be able to increase the efficiency of IVF and embryo transfer to achieve a success rate of at least 50 per cent. This will not be possible if this bill becomes law.

The passage of the bill would deny many thousands of couples in Britain (and 1 in 10 couples are infertile) the means to produce a family of their own, but this is not the only reason why a recent leading article in this journal (*Nature* 7 February, p. 417) described this bill as "home-made and half-baked" and a "blunderbuss" of a bill. Unfortunately, the bill will also prevent attempts to circumvent the production and transfer of severely genetically abnormal embryos, a possibility that is almost within the grasp of medical science, and which is ignored or rejected by proponents of the bill.

The Pro-Life group have lobbied all members of the British Parliament and organized petitions in support of the bill. Last month, Professor Jerome Lejeune was imported from Paris to address the Parliamentary Pro-Life group, and his views on curing inherited genetic disease[1] have been frequently quoted, misquoted and embellished, in support of the group's aims.

It is claimed that severely mentally retarded children with the fragile-X syndrome show improved mental development following treatment with folic acid; that azacytidine treatment of patients with β-thalassaemia may provide an effective therapy for this, and indeed other, inherited diseases; that vitamin therapy to the mother will drastically diminish the risk of spina bifida, and that these examples illustrate that experiments on human embryos are not required. They do no such thing. In fact, they are something of a red-herring, but before dismissing them we should at least look at the facts.

Therapy

The fragile-X syndrome afflicts some 1 in 1,000 males with mental retardation[2]. One diagnostic feature is the presence of a constricted and fragile region at the end of the X chromosome which is visible in a proportion of cells cultured in a medium deficient in folate. Adding folate prevents the cytological expression of this site[3]. This fragile site and the locus causing this form of mental retardation are closely linked, but it does not follow that they are one and the same locus. Indeed, the fragile site is not always readily demonstrable in males apparently afflicted with this form of mental retardation[4,5], while some mentally normal males possess a fragile X chromosome[6].

Folate deprivation also reveals reversible fragile sites on many other chromosomes[7], and they are not associated with mental retardation. The use of folic acid as a treatment for X-linked mental retardation has nevertheless been attempted in a number of small, and often uncontrolled, trials. Subjective "improvements" in the behaviour of some, but not other, folate-treated children have been reported, but with borderline[8] or no[9,10] changes in IQ. The results of these trials are unimpressive.

The proposed treatment for β-thalassaemia raises other questions. 5-Azacytidine (5-AZ) may be incorporated into DNA in place of thymine, but, unlike thymine, it cannot be methylated. Thus, in the presence of 5-AZ, genes whose functions are normally suppressed by methylation may become active[11]. In man, the genes coding for fetal γ-globin become methylated and turned off ("inactivated") after birth and their role in haemoglobin formation in the adult is taken over by the β-globin genes. So in patients with β-thalassaemia, where the genetic defect results in the absence of adult β-globin, one approach for therapy would be to maintain, or restore, the activity of the fetal γ-globin genes to compensate for the absence of functioning adult genes.

In 1982, Ley *et al.* reported[12] that a seven-day 5-AZ treatment of an adult patient with β-thalassaemia resulted in a temporary increase in the level of a fetal γ-globin. 5-AZ is, however, a very dangerous drug; it is likely to be carcinogenic and it also activates latent viruses. Its use would almost certainly be regarded as unethical in the United Kingdom. Although the rationale behind this possible therapy is clear, the longer-term consequences are not. Certainly more than the γ-globin DNA sequences would be expected to be "turned on" in proliferating cells and this kind of treatment could have bizarre, even lethal, consequences.

The third example quoted by the Pro-Life lobby is different in that it relates to a treatment of the mother who bears the fetus and is a preventative therapy. The possible role of vitamin supplementation in reducing the incidence of neural tube defects has been rehearsed in these columns before and will not be repeated. We might note, however, that the Medical Research Council has set up a "multicentre vitamin suplementation trial" to which the Pro-Life lobby has objected.

Understanding

Inherited defects contribute substantially to human ill health and considerable efforts are being expended in attempts to understand their nature and cause. This work very largely involves the use of cultured human somatic cells and its fruits could, in practice, be applied in various ways.

Defining and understanding the nature of the defect can lead to rational attempts to alleviate the disease, by supplementation (*à la* Lejeune) or by circumvention. Attempts at alleviation could, in theory, be undertaken at any stage in development. The widely publicized approach of somatic cell gene therapy in the late fetus or adult, using retroviral or plasmid-based expression vectors[13], and the recombinant DNA research that underpins it, have been the subject of much emotional debate[14].

Yet these developments are some way removed from practicality and, it should be emphasized, will be applicable to only a limited number of diseases. Moreover, they are concerned with attempted amelioration of the disease and not with its prevention. And they involve manipulation of the patient, not of the embryo.

Lejeune has made the point that research aimed at alleviating the symptoms of mental defects, as in Down's syndrome, cannot be performed on early human embryos, because at that stage the central nervous system has not even begun to develop. Similarly, he claims that research on the alleviation of muscular dystrophy cannot be carried out before the muscles are present. These are valid points, because Lejeune is concerned only with therapy. What Lejeune omits to say is that research on the detection and prevention of these genetic defects, and studies designed to increase our understanding of their aetiology, not only *can* be done on the pre-implantation conceptus (early embryo), since the defective genes or chromosomes are present throughout development, but in some instances can *only* be done at this early stage.

Unfortunately, some members of the Pro-Life lobby confuse the attempted treatments of patients with an inherited disease using genetic manipulations of the somatic cell, which may well be a desirable approach, with genetic manipulation of the embryo, which is not only clearly undesirable, but also unnecessary. With severe single-gene inherited disease, 50 per cent of the germ cells of carriers are normal. For an autosomal recessive disorder such as cystic fibrosis, 3 of 4 fertilized eggs produced by two carrier parents (around 1 in every 22 people in the United Kingdom are carriers of this abnormal gene) will result in normal children; for a carrier female of an X-linked recessive gene, as in Duchenne muscular dystrophy, all embryos destined to be female and one-half of those destined to be male (or 3 of 4) will be normal. A fertile carrier of a gene for an autosomal dominant disease such as Huntington's chorea will transmit that gene to one-half of his or her conceptuses. In all cases a proportion of the embryos will result in normal children. Provided we can detect which is normal and which abnormal, there is no need for genetic manipulation of the embryo.

Therefore, it is evident that if we could monitor for the presence of an abnormal gene of chromosomal constitution in an early embryo, then by the use of IVF and embryo transfer it would be possible to ensure that only normal embryos are transferred back to the mother in those families that are carriers of serious genetic disease. In the same way, infertile couples who partake of IVF and embryo transfer or those that are at risk of transmitting a chromosome anomaly, could be assured that the transferred embryos are chromosomally normal. (The first published announcement of the birth of a chromosomally abnormal child, a Down's syndrome baby, conceived by IVF and embryo transfer, has recently been reported[15].) Is it possible to carry out such monitoring? At present, the answer is no. But the means to undertake monitoring are just around the corner, and would require further studies which would become outlawed under Mr Powell's bill.

The chromosome constitution of an early human embryo can be established from a cytogenetic analysis of but a few cells, and such studies have already revealed a high incidence of chromosome abnormality in human embryos[16]. The possibility exists of determining the chromosome constitution of an 8-celled or 16-celled conceptus by removing one or two of its cells and culturing them for chromosome analysis. The conceptus would in the meantime be frozen and afterwards thawed out and transferred back into the woman only if the cells were shown to be normal. (The cells of the early conceptus are multipotential and it has already been shown that a frozen 8-celled human conceptus which has lost some of its cells can give rise to a normal fetus[17].)

The same approach could be used to detect the presence of specific abnormal genes. More and more DNA probes for genes responsible for severe genetic disease in man are becoming available, and further advances in technique will enable such genes to be detected in small cell samples. The means of preventing the transmission of certain severe inherited diseases (with, incidentally, a concomitant reduction in the requirement of abortions in the "at-risk" families) is therefore almost to hand, but their development and implementation will not be possible if the bill succeeds.

Infertility

It is already evident that the very high rate of chromosomal non-disjunction in our own species is responsible not only for many of the genetic defects at birth, but also for much infertility and early spontaneous abortion. We know nothing of its causes, but we do know that much of it arises during oocyte maturation, or during the fertilization process or the early cleavage divisions. *In vitro* studies of these stages of development could therefore throw light on the aetiology of chromosomal disorders.

It is in the field of infertility that research on human oocytes fertilized *in vitro* is most urgently needed and will have the most immediate impact. Already there are hundreds of couples in Britain who owe their babies to such research. The current inefficiency of IVF and embryo transfer can be rectified only if systematic studies of the variables involved can proceed. For instance, the nutritional conditions provided for the fertilized egg *in vitro* are almost certainly not optimal. The IVF conceptus has been shown to secrete chorionic gonadotropin[18], a hormone thought to be crucial for the success of implantation. Conditions of IVF that gave a conceptus secreting low levels of the hormone might well be suboptimal from the point of view of producing a pregnancy.

Male infertility is almost as common as female infertility, but is less tractable to treatment. IVF has proved useful in some cases, since fertilization *in vitro* can be achieved with relatively small numbers of sperm, but the techniques are primitive and more research into the fertilization process itself is needed.

Contraception

A further area in which IVF is already contributing is in the development of new contraceptives. Present contraceptive pills have some undesirable side effects, and attempts to develop a "male pill" have been fraught with problems. The ideal hormonal contraceptive would affect only the oocyte or the sperm and such compounds are now being developed. However, before they could be considered for use it is essential to know if they carry a risk of abnormal fertilization — this can only be ascertained through IVF using human gametes.

In an issue as important and as emotional as this, it is essential that our representatives in Parliament understand the consequences of the proposed legislation. Mr Enoch Powell's bill would make it a criminal offence for any human oocytes to be fertilized *in vitro* except for purposes of "embryo insertion". The endeavours of doctors and scientists to use IVF studies to reduce the numbers of spontaneous abortions and children born with genetic defects, to reduce the numbers of abortions induced on genetic grounds, to alleviate infertility and, by improving contraceptive measures, to reduce the rate of "social" abortions and the numbers of unwanted children — all these would cease. To call such a bill the "Unborn Children (Protection) Bill" seems a prime example of Orwell's Newspeak. □

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1 Lejeune, J in *Test Tube Babies — A Christian View*, 27-36 (Becket Publications, Unity Press, London, 1984)
2 Herbst, D S & Miller, T R *Am J med Genet* 7, 461-469 (1980)
3 Sutherland, G R *Am J hum Genet.* 31, 125-135 (1979)
4 Jacobs, P A *et al Am J med Genet* 7, 471-489 (1980)
5. Froster-Iskenius, U , Schulze, A & Schwinger, E *Hum Genet* 67, 419-427 (1984)
6 Daker, M C , Chidiac, P , Fear, C N & Berry, A C *Lancet* i, 780 (1981)
7 Sutherland, G R , Jacky, P B , Baker, E & Manuel, A *Am J hum Genet.* 35, 432-437 (1983)
8 Lejeune, J , Rethore, M O., de Blois, M C & Ravel, A *Annls Genet* 27, 230-232 (1984)
9 Carpenter, N J , Barber, D H , Jones, M , Lindley, W & Carr, C *Am J hum Genet* 35, 82A (1983)
10. Gilgenkrantz, S , Boue, J., Gregoire, M J & Tejada, I *J Genet Hum* 32, 199-207 (1984)
11 Jones, P A & Taylor, S M *Cell* 20, 85-93 (1980)
12 Ley, T J *et al New Engl J Med* 307, 1469-1475 (1983)
13 Anderson, W F *Science* 226, 401-409 (1984).
14 Culliton, B J *Science* 227, 493-496 (1985)
15 Seppälä, M *et al World Collaborative Report on* In Vitro *Fertilization and Embryo Replacement Current State of the Art in January, 1984 (Ann N Y. Acad Sci.*, in the press)
16 Angell, R R., Aitken, R J , van Look, P F A , Lumsden M A & Templeton, *A A Nature* 303, 336-338 (1983)
17 Trounson, A & Mohr, L *Nature* 305, 707-709 (1983)
18 Fishel, S B , Edwards, R.G & Evans, C *Science* 223, 816-818 (1984)

Is gravitational energy real?

The long-recognized problem of Einstein's theory of gravitation, that gravitational energy is not unambiguously defined, may be unavoidable. But people keep trying.

BETWEEN Newton's time and the beginning of the twentieth century, most people were broadly speaking content with the notion of the potential energy of an object in a gravitational field. Following the Galileo legend, one had merely to drop a stone from a height to see its potential energy converted into kinetic energy. By the nineteenth century, the interplay between the two had become a way of solving practical problems, most elegantly by means of hamiltonian mechanics, but more crudely (as with a falling stone) by equating decreases of potential energy with concomitant increases of kinetic energy. But Einstein changed all that.

It is paradoxical that the best theory of gravitation yet has thrown gravitational potential energy into a confusing limbo. R. Penrose, in a recent paper (*Proc. R. Soc.* **A381**, 53; 1982) puts the point directly, if inelegantly, by noting that "it is perhaps ironic that energy conservation, a paradigmatic physical concept arising initially from Galileo's (1638) studies of the motion of bodies under gravity...should nevertheless have found no universally applicable formulation, within Einstein's theory, incorporating the energy of gravity itself". But people keep trying to define gravitational energy. The latest attempt of this kind, by D. Lynden-Bell and J. Katz (*Mon. Not. R. astr. Soc* **213**, 21; 1985), is intriguing chiefly as an illustration of the difficulties.

The underlying problem with gravitational potential energy in general relativity stems from the principle of equivalence, which logically enters the theory as an assumption, and which has as one of its consequences that an observer falling freely in a gravitational field will have the illusion that the field does not exist. In other words, it is always possible to get rid of a gravitational field by the choice of a suitably accelerated frame of reference. The principle of equivalence leads to the conclusion that free-falling objects always have the same acceleration (which is Galileo's observation). But if gravitational acceleration can always be transformed away, gravitational potential energy cannot have the significance attributed to it in classical mechanics.

Einstein naturally took up the question of how the classical conservation laws would be transferred into his new formalism. One of the frustrations that quickly became apparent is that this seemed not to be unambiguously possible, at least in the strict sense that the representation of a physical quantity should be independent of the coordinates in which this is done (or, technically, that its numerical value at any point should transform with changes of coordinates as does a tensor quantity).

The situation is really rather odd. What Einstein's general theory does is to relate the quantities describing the curvature of four-dimensional space (and which themselves are functions of the space and time coordinates) to a quantity called the energy-momentum tensor, a true physical quantity (in the sense of being a tensor and thus coordinate-free) which represents the energy and the momentum locked up both in matter and in fields other than the gravitational field, electromagnetism for example. Einstein's problem, in the years succeeding 1917, was to find some way of representing the total energy of a system — matter-energy, the energy of the electromagnetic field and of other fields as well as the energy of the gravitational field, together with the modern analogue of the old potential energy — so as then to show that this quantity is conserved.

Einstein recognized that his solution is not ideal. The best candidate for a conserved quantity representing energy is not strictly a tensor but, a construct from the quantities describing the curvature of space-time which is not a tensor in the strict sense but; a pseudo-tensor, *viz* a quantity depending on the coordinate system is Einstein's pseudo-tensor, not a quantity that exhibits on a microscopic scale the conservation of energy and of momentum.

So is there a buried scandal in general relativity? Einstein's failure (often repeated) to find a quantity representing energy and momentum whose microscopic conservation is self-evident does not imply that energy and momentum are not macroscopically conserved. The difficulty that immediately raises its head is that the verification of macroscopic energy conservation must necessarily be possible only within the framework of a particular macroscopic description of the Universe. And many model universes in which energy is not conserved, which are nevertheless inconsistent with energy conservation, have been constructed.

Are there some circumstances in which the definition of potential energy, or the energy of the gravitational field, is possible in terms of the variables that define that field? This is the path that Lynden-Bell and Katz have followed. Beginning with an observation due to J.A. Wheeler that the problem of the unambiguous definition of potential energy may be simpler in the conditions in which spherical symmetry applies, they seek to define the gravitational field energy unambiguously in such circumstances.

The argument goes like this. In a system in which there are only gravitating masses, there must be some quantity, a function of the space-time coordinates, whose integral over any space-like surface (say that corresponding to the present time) is equal to all the energy associated with the mass, its rest-energy and momentum, and its gravitational potential energy. The equations of general relativity allow calculation of the rest-energy from the descriptors of the curvature, so that the difference between the surface integral and the mass within the surface must be the gravitational potential energy. Thus, by considering the difference between the surface integral on two nearby space-like surfaces, it should be possible to define the density of gravitational potential energy, the field energy. Lynden-Bell and Katz pin down their calculation by making an artificial cut in space-time along some space-like surface in which, without loss of generality, they can suppose that space-time is flat.

Physically, the result makes sense. The energy locked up in the gravitational field (as distinct from the mass itself) does indeed decrease with distance from massive objects. Significantly, the field around a black hole turns out to contain the whole energy of the condensed object. This fits in well with the views of those who hold that the significant consequences of black holes are external. Lynden-Bell and Katz say that their result differs from that of the "Penrose school", a reference to Penrose's elegant generalization of general relativity to accommodate particles with spin. That, according to Lynden-Bell and Katz, puts all the energy of a black hole inside it.

The implications of this calculation will depend on whether it can be generalized to universes that are neither spherically symmetrical nor static. There seems to be good reason for hoping that something can be done. But if part of the essence of general relativity is that potential energy cannot be unambiguously defined except by reference to large-scale structure, there is bound always to be a degree of arbitrariness in the way in which the concept arises.

John Maddox

Astronomy

Gas in elliptical galaxies

from A.C. Fabian

IT HAS generally been held that early-type galaxies — lenticulars and ellipticals — are devoid of gas[1]. The gas lost by stars, both as the winds from red giants and as planetary nebulae, has been presumed to be blown out of the galaxies by the cumulative effect of supernova explosions. A galactic wind thus scours a galaxy clean of gas, explaining why only relatively minute quantities (and little star formation) have been detected at radio, optical or ultraviolet wavelengths. This picture, itself, is now being blown away by evidence from the *Einstein Observatory* satellite: a considerable number, and perhaps all, early-type galaxies contain large quanities of gas that are only detectable by their X-ray emission. A new compilation of the data[2] shows that the mass of gas in a large elliptical galaxy can be about the same as that in our own spiral Galaxy.

One of the first results[3] reported from the *Einstein Observatory* was that the Virgo cluster of galaxies appeared 'spotty' in X rays. Individual normal and early-type galaxies appeared as diffuse sources of X rays. Further work[4-6] showed that many other normal galaxies were also X-ray sources, and attention seems to have drifted away from the early-type galaxies to more exotic objects such as active galactic nuclei.

Spiral galaxies, such as our own and Andromeda (M31), are X-ray luminous because they contain populations of X-ray emitting binary star systems. Large elliptical galaxies, however, are about one hundred times more X-ray luminous than spirals, such that over 1,000 very bright X-ray binary sources would be required. That interstellar gas is a more likely candidate for the observed X-ray emission is demonstrated by the X-ray emitting 'plume' of NGC4406 (M86; ref. 3). This large elliptical galaxy is falling through the Virgo cluster at about 1500 km s^{-1} and much of its gas is being stripped out by interaction with the extended halo around M87, to form a tail or plume in its wake. The nearby active galaxy Centaurus A also shows diffuse X-ray emission[7] (apart from the nucleus, jet and so on) which is most likely due to a hot interstellar medium, and not binary X-ray sources. The gas in elliptical galaxies should be at X-ray temperatures, for gas lost from stars moving at speeds σ, relative to the galaxy, of a few hundred km s^{-1} should themalize to a temperature of between 10^6-10^7 K such that its sound speed is about σ.

W. Forman, C. Jones and W. Tucker have compiled[2] the *Einstein Observatory* X-ray data on 55 early-type galaxies, most of which show X-ray coronae, and have obtained results that are of considerable significance to studies of early-type galaxies. Crude spectral analysis shows that the emission is consistent with that from hot gas and not with that of known classes of X-ray binaries. Thus, most optically luminous lenticular and elliptical galaxies contain hot gas; they cannot blow winds. Supernova heating, which could have ejected the gas, must be much less than previously assumed.

An important application of these hot gaseous coronae is the measurement of the masses of early-type galaxies. If the gas is observed, then its bulk motion must be highly subsonic, otherwise impossibly large sources of mass are required to maintain a detectable density. Consequently the coronae must be close to hydrostatic equilibrium and

$$\frac{dP_{gas}}{dr} = -\varrho_{gas}\frac{GM(r)}{r^2}$$

Measurements of the X-ray surface brightness and temperature profiles yield the gas pressure, P_{gas}, and density, ϱ_{gas}, and thus the mass as a function of radius $M(r)$. The compiled data yield total masses of about $1-5\times10^{12}\,M_\odot$ out to 100 kpc for the best studied cases. Some of these galaxies are in the Virgo cluster but most are in the weak groups and some are relatively isolated. The X-ray data imply massive dark haloes around most elliptical galaxies. Their mass-to-light ratio is around 100 in solar units.

For detectable amounts of hot gas to accumulate in galaxies the gas must be cooling at a significant rate, already discussed in the context of a small subset of the present sample[8]. Gas must be cooling and objects condensing out of the resultant cooling flow at a rate of about one solar mass per year, so that the formation of stars in large elliptical galaxies take place at a rate not much less than it does in our own Galaxy. Their optical appearance, however, provides no indication for this. The conditions must be such that the high-mass stars that we normally associate with regions of star formation in our Galaxy are absent. The new stars in eliptical galaxies must be of very low mass, perhaps even below that for nuclear burning. We conclude that elliptical galaxies are not quite so full of old stars as previously thought, although one solar mass per year for a Hubble time does not yield a $10^{12}\,M_\odot$ galaxy. Nevertheless there are intriguing implications for the formation of galaxies and of the so-called dark matter associated with them.

We thus have an extremely powerful tool for measuring the mass profiles out to distances of 10 - 100 kpc and for studying the gas and energy flows in early-type galaxies. When are we going to see more data? Unfortunately, the *Einstein Observatory* re-entered the atmosphere three years ago and the telescope on the EXOSAT satellite is not sensitive enough to help out. ROSAT, to be launched in 2 - 3 years time, should detect many elliptical galaxies out to 100 Mpc or more and, in the 1990s, high-quality imaging X-ray spectroscopy should become routinely available for any elliptical galaxy. □

1 Faber, S M & Gallagher, J S *Astrophys J* **204**, 365 (1976)
2 Forman, W , Jones, C & Tucker, W Center for Astronomy preprint No 2096
3 Forman, W *et al Astrophys J Lett* **234**, L27 (1979)
4 Long, K & Van Speybroeck, L in *Accretion-Driven Stellar X-ray Sources* (ed Lewin & van den Heuvel) (Cambridge University Press, 1983)
5 Bechtold, J *et al Astrophys J* **265**, 26 (1983)
6 Biermann, P & Kronberg, P *Astrophys J Lett* **268**, L69 (1983)
7 Feigelson, E D *et al Astrophys J* **251**, 31 (1981)
8 Nulsen, P E J , Stewart, G.C & Fabian, A C *Mon. Not R astr Soc* **206**, 185 (1984)

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Congenital diseases

Complementary genes for an adrenal enzyme deficiency

from Ian R. Phillips and Elizabeth A. Shephard

ONE of the most common inborn errors of metabolism, congenital adrenal hyperplasia, results from a deficiency in the adrenal cortex of the enzyme 21-hydroxylase[1], which is involved in the biosynthesis of cortisol, the principle glucocorticoid hormone of the adrenal. The disease is inherited as a recessive trait and is closely, but enigmatically, linked to the major histocompatibility complex of humans, *HLA*. Two separate reports now help explain the linkage. First White *et al.*[2] show that the disease is due to a defect in a gene coding for cytochrome P- 450_{C21}, a protein that is specifically catalytic for the 21-hydroxylation of steroids. Second, Carroll *et al.*[3] have shown that the P- 450_{C21} genes lie within the HLA region of human chromosome 6.

Although the biochemical basis of the 21-hydroxylase deficiency has never been definitively established, steroid 21-hydroxylation can be catalysed *in vitro* by a combination of two microsomal membrane proteins of the adrenal cortex: P-450_{C21}, and a NADPH-dependent cytochrome P-450 reductase[4]. Since only the P-450_{C2} is specific for the reaction, White *et al*

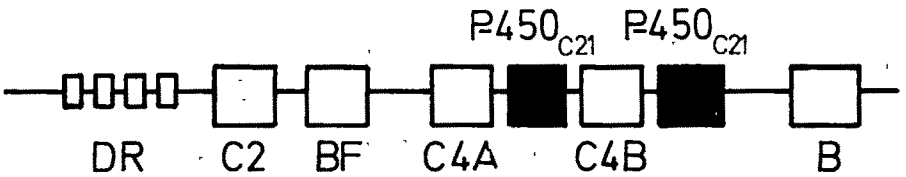
hypothesized that the HLA-linked defect involves a structural gene for this protein. Their test of that hypothesis was facilitated by the knowledge that different forms of the disease are associated with characteristic HLA-B;DR antigens, and that 21-hydroxylase deficiency is in linkage disequilibrium with complement allotypes. For instance, all patients with the haplotype *HLA-(A3);Bw47;DR7* are deficient in both the complement protein C4 and 21-hydroxylase. This led White *et al.* to the additional hypothesis that the genes for C4 and 21-hydroxylase are closely linked and that patients with the Bw47 haplotype may have a large genetic deletion or rearrangement affecting both these genes.

This hypothesis was tested by the use of a cDNA clone coding for bovine P-450$_{C21}$ (ref. 5) to analyse the organization of the P-450$_{C21}$ gene in human DNA. Normal individuals are found to have two P-450$_{C21}$ genes. In the only patient homozygous for both 21-hydroxylase deficiency and HLA-Bw47 antigen, one of the P-450$_{C21}$ genes is deleted. Of patients homozygous for the deficiency but heterozygous for *Bw47*, one carries a homozygous deletion of a P-450$_{C21}$ gene whereas the others all have heterozygous deletions. The deletions segregate with the *Bw47* haplotype over three generations. Thus the *HLA-Bw47* associated 21-hydroxylase deficiency is due to a deletion of a P-450$_{C21}$ gene. However, in the majority of cases where the disease is associated with other HLA-B antigens, the defect is presumably due to much smaller mutations, the nature of which remains to be established.

The elucidation of the molecular genetic basis of congenital adrenal hyperplasia will enable prenatal diagnosis at a much earlier stage than is possible with current methods. This is particularly significant because, in principle, it should be possible to alleviate the condition, at least in female foetuses, by treatment of the mother with dexamethasone.

The prediction of White *et al.* that *C4* and P-450$_{C21}$ genes are closely linked is borne out by Carroll *et al.*[3], beginning with the demonstration that a segment of human DNA containing the *C4* gene hybridizes to a 2.4 kilobase messenger RNA from tissue. Two copies of the gene corresponding to this mRNA are found to lie 1.5 kilobases downstream of the genes for the complement proteins C4A and C4B (see figure). The expression of the gene in the adrenal gland, the size of its mRNA and a partial sequence analysis indicate that it encodes a human P-450$_{C21}$. (Using a similar approach, White *et al.*[6] have demonstrated there are two P-450$_{C21}$ genes located immediately downstream of the *C4* gene and the related *s1p* gene of the mouse, suggesting that a P-450$_{C21}$ gene became linked to the major histocompatibility complex, and was duplicated along with an adjacent *C4* gene, sometime before the divergence of mammals).

In contrast to the genes coding for two other complement proteins, C2 and factor B, which lie one kilobase apart, 30 kilobases upstream from the *C4A* genes (see figure) and which probably arose by duplication of a common ancestral gene, P-450$_{C21}$ and *C4* are not similar in sequence. The significance of their close association is not clear and there is no obvious explanation for the presence in the major histocompatibility complex of genes coding for a steroidogenic enzyme.



Schematic map (not to scale) of the *HLA*-region of human chromosome 6 containing the pair of P-450$_{C21}$ genes. C2, C4A, C4B and BF (factor B) genes encode complement components; DR genes encode Class II HLA antigens and B gene encodes a Class I HLA antigen.

The function of the second P-450$_{C21}$ gene is as yet unknown and it would be of interest to establish whether the two P-450$_{C21}$ genes are highly conserved. It will also be interesting to discover the mechanism responsible for regulating the differential expression of the *C4* and P-450$_{C21}$ genes in liver and adrenals.

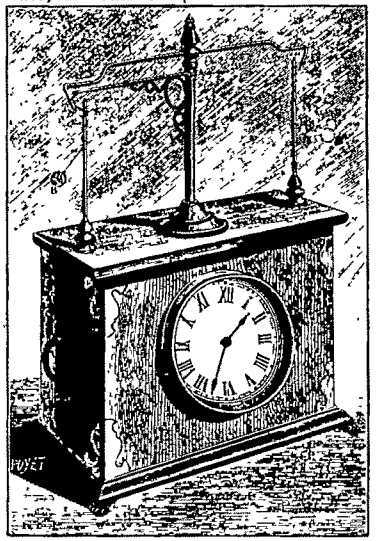
The 21-hydroxylase involved in congenital adrenal hyperplasia is a member of a 'superfamily' of proteins termed cytochromes P-450. Some members of this family, such as P-450$_{C21}$ itself, are involved in the metabolism of endogenous compounds, whereas others play a central role in the metabolism of foreign hydrophobic compounds, including drugs and carcinogens. Three cytochromes P-450 involved in the metabolism of foreign compounds have been mapped to chromosomes 7 and 9 in mouse[7,8] and genes coding for a drug-inducible cytochrome P-450 (ref. 9) have recently been mapped to chromosome 19 in man (E.A.S., I.R.P., M. Davis and S. Povey, unpublished observations). Just as a defect in a P-450$_{C21}$ gene has now been shown to be the cause of congenital adrenal hyperplasia, it is hoped that the availability of cloned DNAs coding for human cytochromes P-450 involved in the metabolism of drugs or chemical carcinogens will lead to a determination of the molecular-genetic basis of inherited variations in the ability of individuals to metabolize these substances. □

1 Kuhnle, U. *et al J clin endocrinol Metab* **52**, 534 (1981)
2 White, P G , New, M I & Dupont, B *Proc natn Acad Sci. U.S A* **81**, 7505 (1984)
3 Carroll, M G , Campbell, R D & Porter, R R *Proc natn Acad. Sci U S A* **82**, 521 (1985)
4 Kominami, S., Ochi, H , Kobayashi, Y & Takemori, S *J biol Chem* **255**, 3386 (1980)
5 White, P G., New, M I & Dupont, B *Proc. natn Acad Sci U S A* **81**, 1986 (1984)
6 White, P G *et al Nature* **312**, 465 (1984)
7 Simmons, D L & Kasper, C B *J biol Chem* **258**, 9585 (1983)
8 Tukey, R H , Lalley, P.A & Nebert, D.W. *Proc natn Acad Sci U S A* **81**, 3163 (1984)
9 Phillips, I R., Shephard, E A., Ashworth, A & Rabin, B R *Proc natn Acad. Sci U S A* **82**, 983 (1985)

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100 Years Ago — A New American Clock

THE accompanying figure from *La Nature* illustrates a new American clock of ingenious construction. It is distinguished from all other clocks by the singular and original form of its pendulum, based on the principal of torsion. A train of wheels gives rotation to a vertical axis, which is seen over the case, the rate of motion of which is to be regulated. This vertical axis supports a sort of bracket, P, to the extremity of which is attached a small bead or ball, B, by means of a thread a few centimetres long. The axis, by the action of the main spring, will turn with a rapid movement, drawing the ball B along with it. To regulate this movement, it is sought to interpose in its path suitable obstacles; this is the object of the horizontal wire terminating in the hooks T, and of the vertical pillars fixed on the case. The bracket P draws the thread in its movement and makes it strike against the arm T; it is thus arrested, and by virtue of its acquired speed, the ball B winds the thread around the pillar on the left; then follows an unwinding of the thread and a rewinding in an inverse direction, which enablesthe thread to pass the point T. But in unwinding it strikes a second time against the pillar, winds and unwinds anew, and only succeeds in passing this double obstacle after four successive windings, twice in one direction, and twice in the opposite direction around the same pillar. The thread thus set at liberty permits the bracket to turn 180° around the vertical axis. After this rotation it encounters two analogous objects placed on the right of the clock, and is delayed a certain time before passing these objects and returning to the pillar on the right. The clock is thus regulated, if not with all the precision of a chronometer, with an approximation said to be sufficient for ordinary use. From *Nature* **31** 439, 12 March, 1885.



Mathematics

How bent is a knot?

from Ian Stewart

Anyone who has fiddled about with one of those lengths of plastic-coated spring from which curtains are sometimes hung will have noticed that it is harder to bend the spring into a knotted loop than it is into a unknotted one. Since elastic energy is concentrated on curves, this suggests that a knot is in general more bent than a simple, unknotted loop. The natural context in which to ask whether any mathematical sense can be made of this observation is differential topology, a hybrid creation in which topological notions, such as knottedness and connectedness, are blended with analytical and differential-geometric ideas such as curvature and smoothness. It is one of the deeper areas of today's mathematics, with important successes and applications to its credit. Recent research by N.H. Kuiper and W. Meeks (*Inventiones Mathematicae* 77, 25; 1984) has uncovered some fundamental relations between the topology of a multi-dimensional space (or manifold) and its metric properties, notably curvature.

The notion of curvature goes back to C.F. Gauss in 1828. He defined curvature for surfaces, proved it was an intrinsic property, not dependent on the specific embedding of the surface in 3-space, and applied it to geodesy. It was generalized by B. Riemann to n-dimensional manifolds, and used by A. Einstein as a basic concept in general relativity. The earliest result relating curvature and topology is the Gauss-Bonnet Theorem: the total Gaussian curvature of a surface is $2\pi C$, where C is its Euler characteristic. This is a topological invariant, equal to $2-2g$, where g is the number of 'holes' in the surface. Roughly speaking, the Gauss-Bonnet theorem says that to make holes in a surface you have to put more bend into it. Its importance may be gauged from a remark in M. Spivak's *Differential Geometry* (a classic 5-volume text, described by its author as "The Great American Differential Geometry Book", published by Publish or Perish Inc.). Spivak says: "A whole slew of consequences follows immediately from this spectacular theorem, which expressed a differential geometric quantity in terms of a number which has nothing at all to do with curvature, or even with a Riemannian metric."

Opening remarks about the curvature of a knot can be made precise in a similar way. In 1949, J. Milnor and I. Fary proved that the total curvature of a knot is greater than 4π. For a planar convex loop, the total curvature is exactly 2π. The ordinary trefoil, or overhand knot, can be embedded in 3-space with a total curvature as close to 4π as required, so 4π is the 'best possible' bound.

Instead of knotting a loop in 3-space, topologists are extremely interested in knotting n-dimensional manifolds in N-space. Again, the intuitive expectation is that a knotted surface will have more bend in it than an unknotted one. The amount of bend is once more measured by the total curvature, which is 4π for a sphere and 0 for a torus (in their natural embeddings). Kuiper and Meeks prove a general result which implies, for example, that a knotted torus in 3-space must have total curvature greater than 16π.

To be specific, they relate the total curvature of an n-dimensional manifold M embedded in N-space, to two topological invariants. One of these depends only on the manifold M: it is a relative of the Euler characteristic, and is the sum, β, of the Betti numbers of M. The other depends on the way M is embedded, and measures the extent to which M is knotted: it is σ_1, the first homotopy excess. (Space limitations preclude a precise explanation of either invariant.) Kuiper and Meeks show that the total curvature must be at least $\frac{1}{2}V(n)(\beta+4\sigma_1)$, where $V(n)$ is the 'surface area' (or total curvature) of the n-dimensional unit sphere. For example $V(1) = 2\pi$, $V(2) = 4\pi$, $V(3) = 2\pi^2$, $V(4) = 8\pi^2/3$. There are, however, two exceptional pairs of dimensions. If $n = 1$ (M is a curve) then 4_1 must be replaced by 2_1. And if $n = 3$, $N = 4$, then ν_1 is replaced by the maximum of two similarly defined quantities, calculated for the interior and exterior of M. In these exceptional cases the results are due to R.H. Fox and P. Wintgen.

The proofs rely heavily on new techniques, including Morse Theory (the study of critical points such as maxima, minima, and saddles), the handle decomposition due to S. Smale, the notion of a tight embedding, and Meeks's work on surfaces known as multipants because of their resemblance to generalized trousers for multilegged creatures. The argument is delightfully geometric, as is most of the deeper work in differential topology.

Much of the power of mathematical reasoning derives from its ability to relate concepts that had not been seen to have any connection with each other. The curvature properties of knotted surfaces are an intriguing example of this process and part of a continuing drive to apply metric concepts, such as curvature, to the topology of manifolds. More detailed results along similar lines cannot be far away; meanwhile the view of Gauss and Riemann that curvature is a fundamental property of a space is being vindicated with a vengeance. □

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Molecular genetics

Diverged genetic codes in protozoans and a bacterium

from Thomas D. Fox

THE genetic code, which is the same in *Homo sapiens* as in *Escherichia coli*, is widely thought to have been fixed prior to the divergence of all life forms and so to be universal. Since a mutation changing a codon assignment would effectively cause thousands of mistakes in the proteins translated, it has seemed quite reasonable to assume that such mutations would be lethal. Although studies on mitochondrial genes have shown that the code is not strictly universal, mitchondria could be thought of as exceptions that prove the rule: their genetic systems produce only a very limited number of proteins and so might tolerate changes. Now, some 'real' exceptions have come to light in both eukaryotic and prokaryotic free-living organisms, and the notion of universality will have to be discarded.

In the erstwhile universal code, the codons UAA and UAG mark the termination of translation of a sequence into protein. But in three different ciliated protozoans there is now a strong indication that UAA and UAG encode the amino acid glutamine. S. Horowitz and M.A. Gorovsky (*Proc. natn. Acad. Sci. U.S.A.* in the press) have determined partial sequences of two genes for histone H3 in the macronucleus of *Tetrahymena thermophila*. (Ciliated protozoans contain a 'somatic' macronucleus and a 'germ-line' micronucleus.) The gene sequences predict the known amino acids of the histone except that each sequence contains two UAA codons at positions that correspond to glutamine in the protein. Thus, UAA must be translated unless both cloned sequences are derived from pseudogenes, which are not translated. This seems unlikely since all 12 mismatches between the two partial H3 sequences produce silent changes. Southern blot experiments show that there are a maximum of four H3 genes in *T. thermophila*, at least two of which must be active since there are two variant forms of the protein in this organism. Thus, while it is possible that the two sequenced H3 genes are silent copies, it is much more likely that UAA actually encodes glutamine.

This conclusion is supported by com-

plementary evidence from another ciliated protozoan, *Stylonychia lemnae*, from which E. Helftenbein (*Nucleic Acids. Res.* **13**, 415; 1985) has cloned two different macronuclear genes that code for a protein which is very similar to the α-tubulins of chicken and rat. However, a UAA codon occurs in both genes at a position equivalent to that where the chicken and rat proteins contain glutamine. Since there are only two detectable α-tubulin genes in the *S. lemnae* genome, it seems that the cloned sequences must be active genes. It is hard to escape the conclusion that UAA is translated as glutamine in the α-tubulin gene of *S. lemnae*, which is terminated by a UGA codon, as are all other genes of ciliates examined to date.

Complementary evidence, reported in this issue from two laboratories, comes from the genes encoding variable surface antigens of *Paramecium*. On page 185, F. Caron and E. Meyer report a partial sequence of the single gene of *Paramecium primaurelia* that encodes the G-antigen. With standard codon assignments, the sequence contains no long open reading frame, but by expression in *E. coli* and sequencing of part of the G-antigen sequence fused to *LacZ*, Caron and Meyer were able to determine the reading frame for the G-antigen sequence in *P. primaurelia*. Interestingly, this frame is the only one of the three possible frames to have UAA and UAG codons but no UGA codons, except for a cluster to three near to the 3′ end of the transcribed region. Moreover, they show that the erstwhile stop codons are present in the G-antigen messenger RNA by using it as a template for sequencing reactions. Analysis of the A-antigen gene of *Paramecium tetraurelia* has led J.R. Preer *et al.* to similar conclusions. As reported on page 188, no long open reading frames are found in the sequences by standard codon assignments, but one frame predicts amino-acid compositions that are generally consistent with analysis of the protein, and this frame contains UAA and UAG, but not UGA codons. While there are no amino-acid sequence data for these surface antigens that allow definite codon assignments, the amino-acid composition data suggest that both UAA and UAG code for glutamine in *Paramecium*.

All of the ciliate genes examined contain the standard glutamine codons CAA and CAG in additon to UAA and UAG. Thus the latter pair could be thought of as resulting from the fixation of a high-efficiency suppressor gene, leaving UGA as the sole termination codon. How, or when, these events took place is anyone's guess. Either the line leading to ciliates diverged before the standard code was fixed or, perhaps more likely, the changes occ urred soon after the divergence of the line from ancestors that used the standard code. This could have happened at a very early stage since ciliates are highly divergent from other eukaryotes: as Horowitz and Gorovsky point out, "Literal use of.. molecular clocks can suggest that ciliates diverged from all other organisms before life originated on earth" (not strictly impossible, but unlikely).

In a prokaryotic organism too, *Mycoplasma capricolum*, divergence of the genetic code has now been demonstrated (Muto, A. *et al. Proc. Japan Acad.* in the press and Yamao, F. *Proc. natn. Acad. Sci. U.S.A.* in the press). This group finds that the sequences of the *M. capricolum* genes coding for the ribosomal proteins S3 and S16, which are highly homologous to S3 and S16 of *E. coli*, contain four UGA codons between them. Three of these occur at positions corresponding to tryptophan in the homologous *E. coli* proteins. Strong support for the idea that these UGAs are translated as trytophan comes from the sequences, determined by the same authors, of two *M. capricolum* genes for tryptophan-tRNA. One contains a standard 5′-CCA-3′ anticodon, but the other has the 5′-UCA-3′ anticodon that would be expected for a tryptophan-tRNA able to recognize UGA.

One cannot help but be struck by the fact that the coding of tryptophan by UGA now demonstrated for one species of *Mycoplasma* is true also of animal and fungal mitochondrial genes, and that some species of *Mycoplasma* can grow as intracellular parasites. So, are *Mycoplasma* closely related to the presumed prokaryotic progenitor of mitochondria? As exciting as this prospect seems, there are at least two reasons for believing it may not be true. First, in at least some mitochondria of higher plants, UGA does serve as a termination codon (A. Brennicke, personal communication), so the use of UGA to code for tryptophan may not have been a property of the common ancestor of mitochondria, if there was one. Second, unlike some other bacterial species which have cytochrome systems strikingly similar to those of mitochondria, *Mycoplasma* appear to lack cytochromes entirely (Pollack, J.D. *et al. J. Bact.* **146**, 907; 1981). It remains to be seen whether non-standard codon assignments will be of value in sorting out phylogenetic relationships or the origins of the genetic systems of intracellular organelles. □

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Laser fusion

Hollow shells, shorter wavelengths

from Roger Evans

SINCE laser-driven inertial confinement fusion (ICF) was declassified in 1972, its somewhat chequered history has centred around the problem of symmetry. Using laser beams, the deuterium-tritium (DT) target pellet has to be compressed to as much as 200 g cm^{-3}, an enormous compression that distinguishes the ICF pellet from the hydrogen bomb. In both cases, the DT fuel must be heated to the thermonuclear ignition temperature of 10^8 K, but the much larger mass of fuel in a bomb need not be compressed to the same degree in order to achieve the combination of density, temperature and duration required to permit fusion — the equivalent of the Lawson criterion. Two recently published papers have shown major advances in solving the symmetry problem and give a renewed vigour to inertial confinement research[1,2].

In the original paper on laser-driven ICF, Nuckolls[3] suggested imploding a small solid pellet of DT fuel, but later Kidder[4] showed that the laser power required could be significantly reduced if the fuel were a hollow spherical shell instead of a solid. For a given fuel mass, this would be larger, its implosion time would be longer, the laser energy could be delivered over a longer time and the power level would be reduced. The extent of this gain depends on the ratio of the shell thickness to its radius, known as the 'aspect ratio'; large thin shells require less laser power to achieve the same degree of compression.

This advantage is not without its drawbacks as the imploding hollow shell suffers from the Rayleigh-Taylor fluid instability. This occurs whenever a dense fluid is supported by a light fluid under gravity. Any distortion of the fluid interface is amplified, resulting in 'spikes' of dense fluid falling while 'bubbles' of the light fluid rise upwards. In laser-imploded targets the gravity is provided by the acceleration, the tenuous plasma heated by the incoming laser light is the light fluid, and the solid shell of imploding material is the heavy fluid. The overall growth of the Rayleigh-Taylor instability scales as the square root of the shell aspect ratio, so that impossible levels of smoothness and illumination uniformity are required for shells of high aspect ratio.

The advantages gained from reducing the laser power with large aspect-ratio shells are not simply the economies of reducing laser costs but are quite fundamental, because light of lower intensity has a more favourable interaction with the target plasma. As the laser intensity increases, a greater proportion of energy goes into removing material from the pellet at the expense of compression efficiency. A major recent advance was the realization that shorter laser wavelengths allow the use of higher light intensities while maintaining good coupling efficiency, because the shorter wavelength light penetrates to

higher densities and thus ablates more material at slower velocity, resulting in more efficient compression. However, coupling the laser energy into high density material reacts back on to the problem of symmetry. No real laser system is ever likely to produce the one per cent symmetry (defined as the fluctuation in laser luminosity over the target's surface) needed to drive a high-density implosion, and some reliance is placed on 'smoothing' the non-uniform illumination within the target atmosphere. Since the short wavelength laser light penetrates further, there is clearly less atmosphere to effect the smoothing and the requirement for illumination symmetry is correspondingly tighter.

The growth of the laser-driven Rayleigh-Taylor instability in flat foil targets has now been measured by workers at the US Naval Research Laboratory[1]. Although this is not the first such measurement[5], it is the first time that two-dimensional images have been obtained. It was made with a flat-foil target, the rear surface of which was grooved like a diffraction grating to act as a 'seed' for the Rayleigh-Taylor instability, accelerated with about 500 J of laser energy at 1.06 μm wavelength. When the instability grows, the line of sight passing through the 'spike' contains much more material than an adjacent sight line passing through the 'bubble' and gives rise to differences in transmission in a flash X-ray radiograph. Comparing the results with sophisticated computer models shows that the growth rate is about half of the classical value. It has been suggested that the reduction is due to the ablation of fluid across the Rayleigh-Taylor unstable region, which gives rise to the classical phenomenon of vortex shedding. If correct, this is good news for short-wavelength laser users because the increased ablation flow should give rise to a further reduction in the Rayleigh-Taylor growth rate.

A second paper[2], from the Osaka group in Japan, reflects the very heavy involvement of the Japanese in fusion research, and particularly in laser fusion, where their Gekko XII glass laser is currently the most powerful in operation anywhere[6]. This group has tackled the problem of uniform illumination in a very imaginative way: instead of trying to achieve the most uniform illumination, use has been made of the paradoxical finding that really bad laser beams can be more effective than good ones, because the target is most sensitive to a particular range of spatial periodicities. Very short wavelength non-uniformities can be removed by the smoothing of the target atmosphere, while wavelengths that are very long compared with the target radius grow quite slowly and are not very damaging.

The Japanese solution is to divide the laser wavefront into a multitude of small rectangular beamlets and to introduce phase delays between adjacent beamlets with a 'random phase mask'. Each beamlet is sufficiently small that, when focused on to the target, its diffraction-limited spot is about equal to the target size; the target sees a large number of diffraction-limited, and therefore smooth, beams. Since the beams are all coherent with each other, they interfere in the target plane and give rise to a scrambled intensity pattern with a very short characteristic wavelength. The Japanese group has performed several target experiments using the random phase mask and has reported favourably on its effectiveness.

The combination of these two new results and other successful implosion experiments reported at the recent International Atomic Energy Agency meeting in London[6] renews enthusiasm for short-wavelength lasers as fusion drivers. Further progress is eagerly anticipated. □

1 Grun, J *et al Phys Rev Lett* **53**, 1352 (1984)
2 Kato, Y *et al Phys Rev Lett* **53**, 1057 (1984)
3 Nuckolls, J M., Wood, L , Thiessen, A & Zimmerman, G *Nature* **239**, 138 (1972)
4 Kidder, R E *Nuclear Fusion* **16**, 3 (1976)
5 Cole, A J *et al Nature* **299**, 329 (1982).
6 Key, M H & Evans, R G *Nature News and Views* **313**, 94 (1985)

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Prehistoric astronomy

Evidence from a new site

from Clive Ruggles

THE ashes of an old and fiery debate on the reality of 'megalithic astronomy' will be stirred by the paper on page 158 of this issue, in which MacKie *et al.* describe, and provocatively interpret, a newly-excavated site in Scotland.

Five years ago it was possible to believe that 'megalithic astonomy' was becoming a sterile field of research. Interest had waned in Stonehenge — for many years the epitome of a supposedly astronomical site — because many highly speculative interpretations, some of which had received wide publicity, had largely been refuted. The long debates generated by one or two high-precision astronomical alignments at a few 'classic' sites, such as Ballochroy in Kintyre and Kilmartin (Temple Wood) in Argyll, were also slackening off, essentially for lack of evidence to assess whether the alignments were deliberate or simply arose by chance.

The meticulous statistical analyses of Alexander Thom, combining evidence from many sites, were then claiming a level of precision for prehistoric lunar observations as little as two minutes of arc, which is quite beyond what seems to be possible, even after observation periods of 200 years. The objectivity of Thom's selection of data was being called into question; and, as a result, so were a number of his claims from earlier analyses, such as the existence of a calendar formed by dividing the solar year into sixteen equal parts, and of indicated horizon foresights marking 'calendrical' declinations.

These claims were starting to be the subject of thorough reassessment by critics from the numerate disciplines, although for many archaeologists this reassessment was redundant. For years they had regarded with grave suspicion the conclusions of astronomers, engineers and the like who, fascinated by the idea of prehistoric astronomy, specifically sought out evidence for it. The dangers in so doing are twofold. First, we may miss or misinterpret a wide range of other evidence bearing upon the purpose of a site and its builders. Second, we may unwittingly project our own ideas of 'achievement' on to the culture whose material remains we are studying. Such ethnocentrism seemed blatant in the approach of those who talked of 'megalithic observatories' — sites considered to have been primarily constructed for careful observation of the Sun, Moon and stars — and led many to dismiss out of hand the conclusions of Thom and others.

Recent developments have changed general attitudes towards prehistoric astronomy. First, there has been a movement away from the consideration of individual sites, towards a more archaeologically-informed assessment of evidence from coherent combinations of sites. Statistical analyses now pay greater attention to the objective selection of data, and the whole approach recognizes that astronomical considerations will be only one amongst a number of factors influencing the placement and design of a site. This sort of approach has uncovered evidence of low-precision lunar alignments amongst linear settings in western Scotland and the Recumbent Stone Circles of Aberdeenshire. At the same time, severe doubt has been cast upon the hypothesis of observations more precise than one or two degrees (including Thom's solar calendar).

Second, archaeologists have begun to consider astronomical practice as but one aspect of ritual activity undertaken at ceremonial sites. Thus by relating evidence of lunar orientation to excavated evidence at the Recumbent Stone Circles, Aubrey Burl has concluded that the sites had a ceremonial significance in which lunar symbolism was important. The increased communication between those trained in the sciences and those trained in the humanities has lain the seeds of more general attempts to study and understand prehistoric astronomy, not in isolation but more properly in relation to its social context.

While statistical analyses must continue to play a crucial role, investigations of particular sites still have something to

offer, particularly if their excavation is designed to test a particular astronomical hypothesis rather than simply to collect data. A long-standing proponent of this approach has been Euan MacKie, who in 1970 and 1971 excavated a structure at Kintraw, Argyll, claimed by Thom to be an observing platform. Although widely quoted, the evidence from the excavation was unfortunately inconclusive, as MacKie himself now admits. In this issue of *Nature* he and his co-authors discuss evidence from Minard — another site in Argyll — where archaeological excavation was seen as testing an astronomical hypothesis. Perhaps the most striking result is that, unlike Kintraw, Minard indisputably has platforms that have been levelled by man; moreover, the main features at the site have been fairly securely radiocarbon dated to the mid-second millennium BC. Together, the various archaeological discoveries indicate that Minard was not a domestic, defensive, industrial or funerary site, but one intended, perhaps almost exclusively, for ritual use. Furthermore, both the striking distant horizon to the north-east, roughly in the direction of midsummer sunrise, and the general orientation of the site in this direction, seems to indicate that solar orientation was a deliberate and crucial function of the site.

Those interested in prehistoric astronomy will still have many questions to raise about the precise interpretation of Minard. In particular, many will dispute whether, on statistical grounds, the claim that it was used for high-precision observations (supporting Thom's 'calendrical' hypothesis) is really borne out. The north-eastern horizon foresight does not fall



Minard, Argyll. Was the site used for astronomy in megalithic times?

exactly at the solstice, and any feature occurring within a few degrees of the solstitial position is susceptible to the 'halving the difference' argument used by MacKie *et al.* (*viz*). There are similar problems when defining the 'calendrical' equinox. Thus the evidence for high-precision astronomy is perhaps not as clear as MacKie's paper might lead us to believe; nonetheless Minard is surely destined to become an important site, evidence from which will generate considerable debate and, I hope, a few fresh hypotheses. □

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Genetic analysis

Of man and mouse

from P.N. Goodfellow

ON page 181 of this issue B. Robert *et al.*[1] describe their determination of the chromosomal localization of the genes that code for several muscle proteins of the mouse. These results will engage those studying co-ordinate regulation of muscle proteins and those interested in the evolution of gene families. More importantly, by exploiting the species differences between *Mus domesticus* and *Mus spretus*, Robert *et al.* introduce a new technique of mapping genes to chromosomes that may well become a standard tool for genetic analysis of the mouse.

The laboratory mouse has many advantages over man for genetic analysis, including a short generation time, plentiful genetic markers, and no restrictions on brother-sister or parent-child matings. So great were the advantages of working with mice that many geneticists believed that the proper study of man was mouse, a view which was encouraged by the development of inbred[2], congenic[3] and recombinant-inbred strains[4]. Without these tools, it is doubtful whether many familiar systems, such as the genetics of transplantation and immune response, would yet have been unravelled.

Two advances have made the direct study of human genetics as profitable as the study of the mouse. First, the introduction of somatic cell techniques freed human genetics from the disadvantage of the long human life span and exploited the species differences between humans and rodents to provide genetic markers; using this method over 400 loci have been assigned, directly or indirectly, to human autosomes[5], whereas only two or three had been assigned before the application of somatic cell genetics[6]. Second, in recent years, molecular biology has provided an almost limitless supply of genetic markers in the form of restriction fragment length polymorphisms (RFLP) defined by cloned DNA probes (see ref.7 for a recent review and ref.8 for the latest advance). These markers have rejuvenated classical genetic approaches and have already contributed to the chromosomal localization of loci associated with muscular dystrophy[9] and Huntington's disease[10].

Mouse genetics has also benefitted from somatic cell techniques and molecular biology but because of the related origin of the common inbred mouse strains[11,12] and the small number of strains available[2], it more difficult in mouse than in man to find RFLPs that can be used as genetic markers. To overcome this deficiency, Robert *et al.* mated an inbred strain of the laboratory mouse, *Mus domesticus*, to an inbred strain of the related mouse, *Mus spretus*[13]. The two progenitors of the laboratory mouse, *Mus musculus* and *Mus domesticus*, have been separated from *Mus spretus* for about three million years[14], which is sufficient time for there to have been enough divergence in DNA sequences to generate potential RFLPs in any cloned mouse gene.

In the wild, *Mus domesticus* and *Mus spretus* probably never mate with each other[15], but, under carefully provided laboratory conditions, fertile female offspring can be produced[13] and back-crossed to the parental strains[16]. (The male offspring of the between-species cross are infertile, as predicted by Haldane's law[17]). As demonstrated by Robert *et al.* for myosin alkali-light chain genes[1], linkage between the RFLPs and other genetic markers can easily be followed in the back cross and used for gene mapping.

For some genetic studies, including deliberate mutagenesis, the mouse will always be the mammal of choice. Ethylnitrosourea can induce mutations at the incredibly high frequency of one mutant at each locus for every thousand offspring[18]. This means that saturation mutation mapping[19] and the deliberate creation of mouse models for human disease are both feasible. Perhaps the proper study of man is both man and mouse. □

1. Robert, B. *et al. Nature* **314**, 181 (1985).
2. Festing, M.F.W. *Inbred Strains In Biomedical Research* (Macmillan, London, 1979).
3. Snell, G.D. *J. Genet.* **49**, 87 (1948).
4. Bailey, D.W. *Transpl.* **11**, 325 (1971).
5. Shows, T.B., McAlpine, P.J. & Miller, R.C. *Cytogenet. Cell Genet.* **37**, 340 (1984).
6. Renwick, J.H. *Ann. Rev. Genet.* **5**, 81 (1972).
7. White, R. *et al. Nature* **313**, 101 (1985).
8. Jeffreys, A.J., Wilson, V. & Thein, S.L. *Nature* **314**, 67 (1985).
9. Murray, J.M. *et al. Nature* **300**, 69 (1982).
10. Gusella, J.F. *et al. Nature* **306**, 234 (1983).
11. Ferris, S.D., Sage, R.D. & Wilson, A.C. *Nature* **295**, 163 (1982).
12. Yonekawa, H. *et al. Differentiation* **22**, 222 (1982).
13. Bonhomme, F., Martin, S. & Thaler, L. *Experientia* **34**, 1140 (1978).
14. Ferris, S.D., Sage, R.D., Prager, E.M., Ritte, U. & Wilson, A.C. *Genetics* **105**, 681 (1983).
15. Britton, J. & Thaler, L. *Biochem. Genet.* **16**, 213 (1978).
16. Bonhomme, F., Benmetidi, F., Britton-Davidian, J. & Martin, S. *C.r. Acad. Sci. Ser. D.* **289**, 545 (1979).
17. Haldane, J.B.S. *J. Genet.* **12**, 101 (1922).
18. Russell, W.L. *et al. Proc. natl. Acad. Sci. U.S.A.* **76**, 5818 (1979).
19. Bode, V.C. *Genetics* 108, 457 (1984).

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Histidine-rich protein of *Plasmodium lophurae*

SIR — Ravetch *et al.* have reported some very interesting findings on the primary structure and genomic organization of the histidine-rich protein of *Plasmodium lophurae*[1]. Their observation of the presence of multiple copies of the signal-peptide sequence in the genome of *P. lophurae*, along with the increasing evidence for the presence of histidine-rich sequences in additional malarial polypeptides, pose numerous challenging questions. The fragmentary available information seems to suggest that some related sequences have been conserved through the evolution of apparently functionally different malarial polypeptides.

The recent literature on histidine-rich proteins of malaria parasites has certainly had inconsistencies and can seem confusing to newcomers to the field. This creates all the more reason for factual reporting. Until 1983, the unique and fully characterized histidine-rich protein of *P. lophurae* was referred to as the 'HRP' of *P. lophurae* in all the malarial literature. Although in my publications a clear distinction has been set between HRP and other related polypeptides, there have been several references to undefined histidine-containing malarial polypeptides as 'HRP'.

Perhaps by confusing fact with hypothesis or different histidine-rich polypeptides, Ravetch *et al.* have taken their interesting findings totally out of the context of *P. lophurae*. They cite my paper[2] as their source for the statement that the histidine-rich protein "is located in a membrane-bounded compartment that forms part of the specialized parasite organelles implicated in erythrocyte invasion". Technically, these specialized organelles are the rhoptries of merozoites, the infective forms. However, the facts presented in the paper cited show that the protein was purified from cytoplasmic granules of trophozoite stages of *P. lophurae*[2]. As the parasites develop, the granules increase in number and eventually become segregated inside the residual food vacuole. When the host cells lyse and merozoites are liberated, the granules remain enclosed in the food vacuole which is also released.

These facts offer no grounds for speculating that "if histidine-rich protein becomes a surface molecule of *P. lophurae*, it must be secreted" or "if secretion does occur, it would be followed by disassembly of protein aggregates before association with the parasite surface". As shown in the electron micrograph of my paper, the protein aggregates in the form of electron dense granules while it is within the rough endoplasmic reticulum. Therefore, the logical interpretation of the observed structural features which are characteristic of secretory proteins has to be sought in the context of the synthesis and translocation of the polypeptide within cytoplasmic compartments of the parasite, rather than secretion to the surface. I have described a secretory polypeptide that is related to the histidine-rich protein of *P. lophurae* but it is in another species of malaria. Also, the idea of the possible relationship of a histidine-rich protein to the infection process does originate from my paper entitled 'Does a histidine-rich protein from *Plasmodium lophurae* have a function in merozoite penetration?'[3]. This was published as a hypothesis and has been neither proven nor disproven. I would be delighted to see it proven.

Ravetch *et al.* seem confident of the accuracy of the tandemly repeated sequence. In comparison with the known amino acid composition of the mature polypeptide, the deduced sequence shows significant differences in proline and aspartic acid content. In our hands, the histidine-rich sequences of both genomic and cDNA clones were totally unstable in *Escherichia coli*.

Finally, the statement that "other investigators have been unable to reproduce" my results on the protective effect of the histidine-rich protein cannot be supported by the literature cited. The use of altered experimental protocols does not imply "reproduction".

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1. Ravetch, J.V., Feder, R., Paulovec, A. & Blobel, G. *Nature* **312**, 616-620 (1984).
2. Kilejian, A. *J. biol. Chem.* **249**4650-4655 (1974).
3. Kilejian, A. *J. Protozool.* **23**, 272 (1976).

Climatic effects of volcanic eruptions

SIR — Major volcanic eruptions in the future will cause, as they have in the past, significant short-term climatic changes, and these changes may have severe social consequences[1-5]. Recent discussions of the climatic effects of volcanic eruptions have emphasized their importance as analogues of the consequences of nuclear war[1,2]. While studies of volcanic eruptions can make an important contribution to assessing the effects of a possible nuclear war, we fear that to use volcanic studies in this way draws attention away from the very serious problems that the eruptions themselves present. A nuclear war remains, fortunately, only a hypothetical possibility. But it is certain that climate-altering volcanic eruptions will take place in the future, although the frequency of these is not well established[6].

The most recent such eruption, that of Tambora 1815, caused widespread disruption to agriculture in North America and Europe, leading to economic crises, famine and outbreaks of disease, whose effects have been summarized by Stommel and Stommel[7]. The stratospheric aerosol loading produced by Tambora was equivalent to a peak optical depth of about 1 (ref. 3). Much the largest effects of historic times appear to have been associated with a volcanic aerosol of unknown provenance of AD 536, calculated to have had an optical depth of about 2.5, which had severe effects on agriculture in the Middle East[4]. Several other historic eruptions have had less severe but still significant effects, notably the Laki eruption of 1783[8].

Since 1815, the world's population has increased many-fold. The continuing famines in the arid and semi-arid regions of northern Africa demonstrate the disastrous human problems that result when relatively minor climatic changes affect marginal areas which are already at or near their maximum sustainable populations. An eruption of the magnitude of Tambora taking place at the present day could have catastrophic effects on agriculture, causing crop failures and shortages world wide, with substantial social and political implications. The fact that the frequency of such large volcanic eruptions may be rather low[3] offers little comfort: a Tambora or even a Toba magnitude eruption could take place tomorrow. Furthermore, the climatic effects of volcanic eruptions are a function of their ability to produce stratospheric aerosols as well as their absolute magnitudes[9,10]. Eruptions smaller than that at Tambora could therefore cause acute problems, especially given the present human pressure on food supplies.

Whilst not wishing to minimize the importance of research on the climatic effects of nuclear war, we suggest that some effort should be diverted from the hypothetical to the inevitable, and that attempts be made to determine the possible climatic and agricultural effects of future large and intermediate magnitude volcanic eruptions. Governments and international agencies might then be in a position to prepare contingency plans capable of dealing with the likely eventualities.

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1. Rampino, M.R., Stothers, R.B. & Self, S. *Nature* **313**, 272 (1984).
2. Turco, R.P., Toon, O.B., Ackerman, T., Pollack, J.B. & Sagan, C. *Science* **222**, 1283-1300 (1984).
3. Stothers, R.B. *Science* **224**, 1191-1198 (1984).
4. Stothers, R.B. *Nature* **307**, 344-345 (1984).
5. Rampino, M.R. & Self, S. *Quat. Res.* **18**, 127-143 (1982).
6. Self, S., Rampino, M.R., Newton, M. & Wolff, J.A. *Geology* **12**, 659-663 (1984).
7. Stommel, H. & Stommel, E. *Volcano Weather* (Seven Seas Press, Newport, Rhode Island, 1983).
8. Wood, C. *Eos* **65**, 410 (1984).
9. Rampino, M.R. & Self, S. *Nature* **310**, 677-679 (1984).
10. Self, S., Rampino, M.R. & Barbera, J.J. *J. Volcanol. Geotherm. Res.* **11**, 41-60 (1981).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

Origin of strong interplanetary shocks

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Observations of 900 radio sources have been used to study the shape and motion of large-scale interplanetary transients associated with shock disturbances at 1 AU. The variations of plasma density and speed and the zones in the solar atmosphere from which the transients originate suggest an origin related to intermittent flows of enhanced speed from coronal holes at mid-latitudes on the Sun.

A BETTER understanding of the mechanisms involved in the genesis of strong interplanetary shocks is important not only to solar physics, but also for practical reasons concerned with disturbances to communication systems, electrical power transmission, geomagnetic surveys and with possible radiation hazards to astronauts[1,2]. New information about the shape and dynamics of large-scale interplanetary transients is now becoming available from radio astronomical measurements on a large grid of 900–2,500 radio sources whose scintillation can be used to monitor perturbations of plasma density along many lines of sight through the interplanetary medium[3]. The ability to map the disturbances and to estimate their speed and direction of travel is helpful in locating their sources at the Sun. Here, we present observations of interplanetary transients associated with three of the strongest shocks that reached the Earth during 1978–79: about 70 other examples were found during this period of high solar activity and will be presented in detail elsewhere.

Plasma density

Our observational methods and techniques of data reduction have been described before[3,4]. For each source we measure ΔS, the r.m.s. variation of flux density at 81.5 MHz integrated over the band 0.1–3.0 Hz during an interval of about 30–50 s at meridian transit. ΔS varies systematically with solar elongation in a manner which can be determined accurately from daily observations continued for about 1 yr. To specify day-to-day perturbations about the mean, we define an enhancement factor $g = \Delta S/\overline{\Delta S}$, where $\overline{\Delta S}$ is the average value for a given source at a particular elongation.

It has long been known that variations of g are correlated strongly with variations of total plasma density along the line of sight to a source[3]. It has also been suggested that enhanced turbulence, rather than enhanced density, may sometimes be correlated with increased scintillation[5]. This possibility is important for the present study, where lines of sight pass frequently through post-shock flows, so we have performed an analysis to check the correlation under a variety of interplanetary conditions. The large grid of 900–2,500 sources under daily observation enables this to be performed more accurately than had previously been possible.

The region of the solar plasma responsible for scintillation at 81.5 MHz is sketched in Fig. 1*a*. On 30 occasions during 1978–81, when we were fairly certain that stable conditions persisted along most of the relevant portions of appropriately located lines of sight, we obtained the results shown in Fig. 1*b*. The observations refer to lines of sight within 30° of the ecliptic at elongations near 90°, so that the best comparison could be made with near-Earth spacecraft data; a full account of this work will be given elsewhere. Occasions were selected when the *in situ* density remained approximately constant for at least 24 h and the average value of g was obtained typically from observations of 50–100 sources. The conditions giving rise to perturbed plasma density included both relatively stable co-rotating streams and more sudden transients often associated with shocks.

Our results are shown in Fig. 1*b*, which demonstrates the strong correlation between plasma density (N) and g. Over the range $3 < N < 40$ cm^{-3} the observations satisfy a relation $g = (N\,\text{cm}^{-3}/9)^{0.52\pm0.05}$. During the 2-yr period near sunspot maximum, we found no evidence for enhanced or decreased scintillation not associated with corresponding variations of density. We therefore conclude that scintillation can be used with confidence to monitor plasma density along the line of sight to a source under widely differing heliospheric conditions.

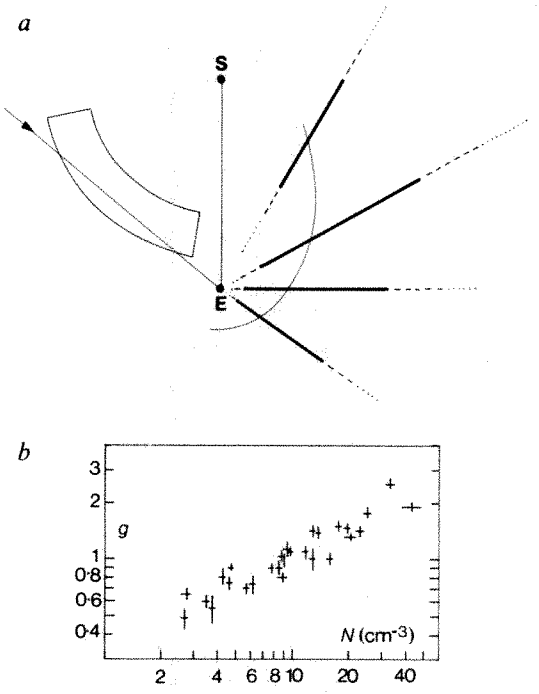


Fig. 1 *a*, Schematic diagram of scintillation weighting functions along various lines of sight normalized to unity at the locus of maximum scatterng (curved line). The ranges are: 0.5–1.0 (heavy line), 0.25–0.5 (dashed line), 0.1–0.25 (dotted). S, Sun; E, Earth. *b*, Correlation of scintillation enhancement factor (g) with *in situ* plasma density N. Each point is the average of observations of 50–100 sources at elongations near 90°.

The technique is best suited to the detection of large disturbances which cover the heavily weighted portions of the lines of sight. Shell-type transients thinner than ~0.05 AU might be undetectable. As our observations are made at meridian transit, there is also a finite chance that some disturbances could pass through the zone of detectability unobserved if they travelled faster than ~1,000 km s^{-1}.

Observations

The transients to be discussed caused sudden-commencement magnetic storms when they reached the Earth, followed by the strongest geomagnetic disturbances (Ap > 100) during August 1978–September 1979 and were also accompanied by energetic particles.

Contour maps showing the variations of g across the sky as the transients propagated outwards from the Sun are shown in

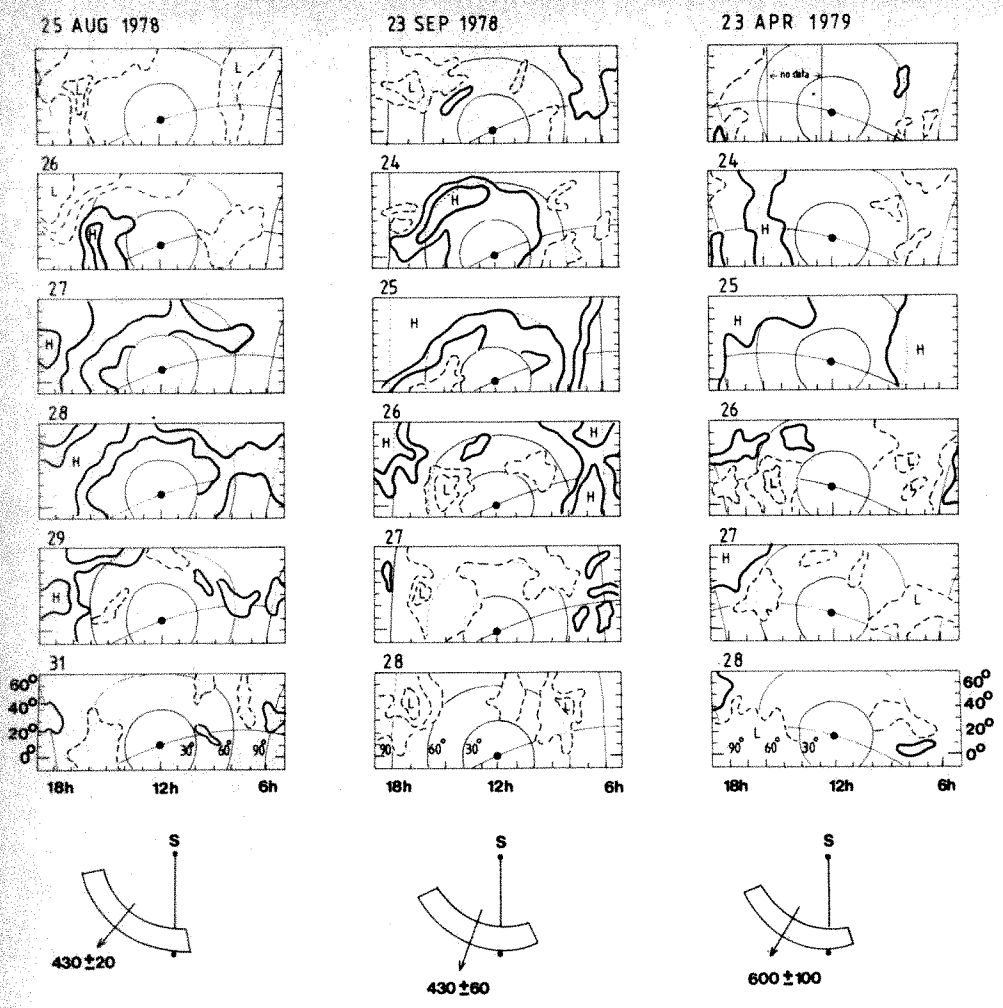


Fig. 2 Sky maps of scintillation enhancement (g) for three transients; coordinates: x, hour angle; y, declination. The contour levels for $g>1$ are 1.25, 1.5, 1.75 (heavy lines) and for $g<1$ are 1/1.25, 1/1.5, 1/1.75 (broken lines). Areas of highest (H) and lowest (L) values are indicated. Approximate shapes and speeds of enhanced density transients derived from the maps are also shown.

Fig. 2. Most of the information about transients is obtained from observations within 1 AU and so the maps have been plotted only to ±7 h from local noon. For qualitative interpretation, note that lines of sight near elongation 90° monitor disturbances as they pass the Earth. Mapping is not continued within 30° from the Sun as this is the zone of strong scattering where g is expected to show an inverse correlation with density. Significant perturbations of g are not usually observed in this region.

We have shown previously how a comparison of observed maps with theoretical maps computed for simple models of density perturbations can be used to deduce the density structure in three dimensions[4,6]. The period 1978–1979 had unusually high solar activity and most of the disturbances that we observed approximated to outward-moving spherical shells or co-rotating density structures associated with relatively long-lived high-speed solar wind streams. The approximate shapes of the enhanced density zones of the three transients are shown in Fig. 2. It is possible that the radii of curvature of the disturbances are less than indicated (although they must exceed 0.5 AU), but there is no doubt that they cover a wide range of helio-longitude and latitude. The typical solid angle is of the order $\pi/2$ sr; the precise latitude range is less easily determined and will be discussed below. The radio source grid is sufficiently fine that the outer boundaries of the transients can usually be located to an accuracy of ±5° and the day-to-day positions can then be used to estimate their speeds and arrival times at the Earth. Some other features on the maps, such as the low-density zones on 25–26 August, the structures at elongations >45° on 23 September and the high-density zone at elongation >90° on 24 April are associated with other transients or longer-lived co-rotating streams and will be described elsewhere.

Figure 3*a* shows the expected position as a function of time of the outer boundary of enhanced scintillation computed for

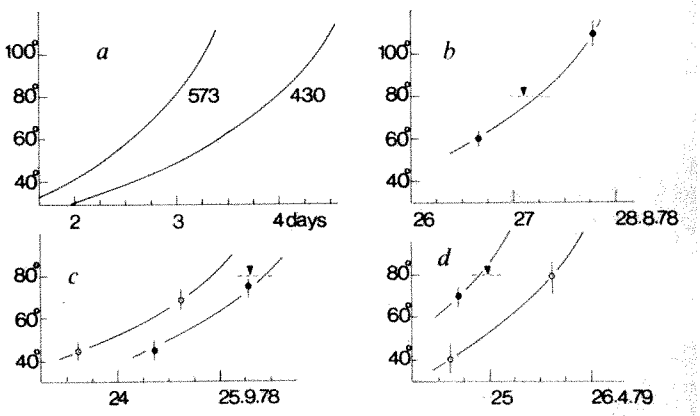


Fig. 3 *a*, Solar elongation of the outer boundary of enhanced scintillation computed for a spherical shell of enhanced density travelling at 30° to the Sun/Earth line. The starting time at the Sun is 0 days. *b*–*d*, Observed positions on the boundaries of transients. *b*, Outer boundary near the ecliptic to the east (●); *c*, outer boundary above the ecliptic (⊙) and on the ecliptic to the west (●); *d*, outer (●) and inner (⊙) boundaries near the ecliptic to the east. The times of sudden-commencement magnetic storms are indicated (▼). The curves fitted to the observations correspond to the speeds shown in Fig. 2.

a spherical shell of increased density of thickness 0.2 AU, covering a range of ±36° in helio-longitude and latitude and centred on a direction at 30° to the Sun/Earth line in the ecliptic. Constant speeds of 430 km s^{-1} and 573 km s^{-1} were assumed, corresponding to transit times of 4 and 3 days from the Sun to the Earth. The weighting of scintillation along the line of sight is such that the curves for elongations <100° are not sensitive

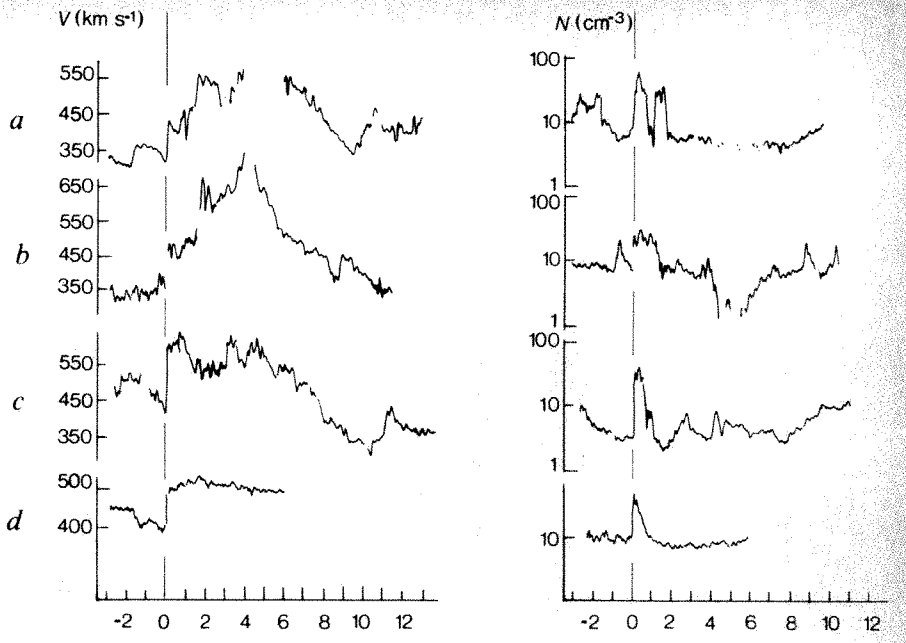


Fig. 4 The solar wind speed and plasma density observed by spacecraft near the Earth. The temporal variations are aligned with respect to the shocks (vertical line) at zero time. The arrival times are: *a*, 0300 UT, 27.8.78; *b*, 0718 UT, 25.9.78; *c*, 2357 UT, 24.4.79; *d*, a superposed epoch plot of 103 shocks observed during 1971–78.

to the direction of travel, provided that the transient is centred on a line within ~45° of the Sun/Earth line. The models indicate that the outer boundary is seen at an elongation near 80° when the transient is about to hit the Earth. Some observed positions of the boundaries of the transients mapped in Fig. 2 are plotted in Fig. 3*b–d*. The measurements refer to positions near the ecliptic, either to the east or west of the Earth. The September transient was clearly directed above the ecliptic and in this case we have also shown the location of the outermost boundary at high helio-latitude. Radio interference prevented observations of the front of the transient on 23 April and here we have made use of positions of the inner boundary. The curves fitted to the points are model curves (as in Fig. 3*a*) with suitable velocity scaling.

The times at which the transients reached 1 AU in the ecliptic are in good agreement with the onset of sudden commencement magnetic storms, indicating that the shock fronts must be close to the outer boundaries of the high-density shells causing enhanced scintillation. To complete the observations we display in Fig. 4*a–c*, the solar wind speed and plasma density observed by spacecraft close to the Earth[7] during the same periods.

Solar origin of the transients

To seek possible sources of the transients, we projected them back to the solar surface assuming transit times of 3 days for August and September and 2 days for the faster April transient. These delays are less than the 4- and 3-day transit times appropriate to our derived speeds and were chosen to allow for slight deceleration in accordance with spacecraft observations of the April event[8] for which the mean speed between 0.41 AU and 0.99 AU was 789 km s^{-1}, whereas the speed at 1 AU was 608 km s^{-1}.

The zones so defined are shown as circles on the synoptic maps in Fig. 5*a–c*. The greatest uncertainty concerns the helio-latitudes, as in two cases the maps do not extend sufficiently far below the ecliptic. It is probable that the August transient was directed slightly above the ecliptic, whereas that of April could have been below. Because this could not be confirmed they have been centred at 0°. Also shown on the synoptic maps are the outlines of coronal holes obtained from He 10,830 Å images (Solar Geophysical Data), the positions of notable solar flares or disappearing filaments discussed in connection with the same transients by other workers and the projected ranges in helio-longitude at the Sun of the high-speed streams ($V >$ 450 km s^{-1}) observed by near-Earth spacecraft[7]. The latter estimates were based on an assumed transit time of 3 days.

Figures 4 and 5 show that the shells of high density are followed by high-speed streams which originate from roughly the same zones on the Sun and subsequently persist for at least 6 days. During the September event the stream showed a further acceleration some 4 days after the initial shock. The same streams are also seen in Fig. 2, where they correspond to zones where $g < 1$, in accordance with the usual reduction of plasma density in regions of higher than average speed. Note that averaging along the line of sight in our data smooths out some of the complexity seen in spacecraft measurements at a single point and also that the density was already low before the April transient. One feature common to all the transients is that they erupted from zones containing coronal holes at moderately low helio-latitude. As it has been established that long-lived coronal holes, such as those that existed during 1974–75, are sources of relatively stable high-speed streams, we believe that our observations are most easily explained by supposing that less stable holes, typical of the ascending phase of the solar cycle[9], are the sources of variable speed flows. If the wind from such a hole increased from, say, 450 km s^{-1} to 600 km s^{-1} on any timescale shorter than ~12 h, the faster flow would catch up the slower flow ahead of it, thus generating a compression zone of enhanced density and a steepening velocity gradient which could develop into a shock at 1 AU. Theoretical modelling with reasonable velocities has demonstrated the general characteristics of the expected perturbations[10].

The same coronal hole was associated with both the August and September transients. It first appeared on Carrington Rotation 1671 and persisted for three rotations. Clouds prevented observations on 23–24 August when the transient high-speed flow should have commenced but a significant increase of area (~25%) occurred during 22–25 August (J. W. Harvey, personal communication). Such changes are fairly common and might signal increases of solar wind speed. There are two possibilities for the source of the April transient which has been associated previously[8] with the disappearing filament shown in Fig. 5*c*: (1) the hole at longitude 30° and latitude 25°N; (2) the complex hole structure in the Southern Hemisphere. On the following rotation (CR 1681) a large hole at similar longitude extended upwards from the southern pole to latitude 15°S following the tendency for mid-latitude holes to merge with holes at higher latitudes as they overtake them under differential rotation[9]. Coronal hole activity in the south is the most probable source of the transient.

Precise alignment of the projected zones of origin with coronal holes is not expected. High-speed streams at 1 AU typically extend over wider ranges of longitude than those of the hole

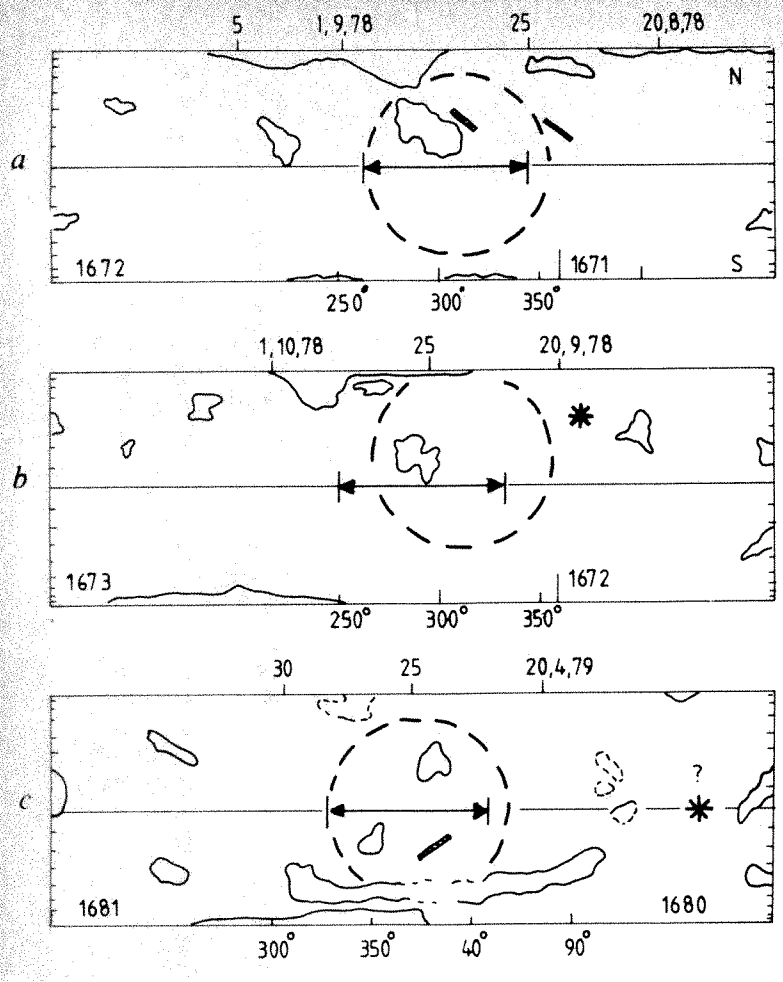


Fig. 5 Predicted areas (dashed circles) from which three transients erupted plotted on synoptic maps showing the boundaries of coronal holes obtained from He 10,830-Å images. Arrowed zones on the Equator denote the predicted longitude range of high-speed streams of speed ≥450-km s^{-1}. Date marks correspond to CMP at 0000 UT. The locations of flares (stars) and disappearing filaments are indicated.

boundaries seen in the He 10,830-Å images and little is known about the divergence of streams near the Sun. Again, the co-rotation of transient high-speed streams after their eruption will generate enhanced density at the western edges of the streams[11]; this could account for the small (and possibly insignificant) systematic westerly displacement of the high-density zones with respect to the streams seen in Fig. 5.

Sudden disappearances of solar filaments have been associated with the transients of August and April and their positions are shown in Fig. 5 (refs 8, 12 and J. W. Harvey, personal communication). Both the timing and location of these events are consistent with our observations; it is reasonable to speculate that large-scale disturbances of the solar wind will be accompanied by major changes in the coronal magnetic field that could destabilize the filaments.

Solar flares have also been associated with two of the transients[13] although that for the April event was later discounted[14]. Our observations disagree with the suggested flare-source for the September transient, shown in Fig. 5*b*, where there is a large discrepancy in position and the timing is also inconsistent. The flare occurred at 0940 UT on 23 September while the outer boundary of the transient was observed at a distance of 0.7 AU at 1500 UT on the same day. If the flare triggered the disturbance, the average speed must have been ~7,000 km s^{-1}, which is unreasonably high.

We discussed previously another flare discrepancy[3] and our observations during 1978–81 contain further examples, raising the question of whether flares are as effective in causing shock-disturbances at 1 AU as is sometimes assumed[13]. Figure 4*d* shows a superposed epoch plot of 103 shock events at 1 AU, the sample constituting all the shocks observed during 1971–78 on the IMP 6, 7, 8 spacecraft[15]. The average properties are very similar, though less extreme, than the major transients we have described. In particular, the high-speed flow behind the shocks lasts, on average, for 5–6 days. Both the duration of the flows and the slow increase of velocity to a peak 1–2 days after shock passage are more similar to the behaviour of transient solar wind streams than to the expected properties of shocks induced by the sudden release of large amounts of energy in the lower corona[16]. In the latter case, models predict a swifter return to ambient conditions.

The new cause that we suggest for major disturbances does not eliminate the possibility of correlations between flares and shocks at 1 AU. It is probable that the emergence of more energetic solar wind flows is accompanied by changes in the coronal magnetic field structure which could trigger a variety of sudden events in the solar atmosphere including flares and eruptive prominences. But these would be peripheral to the main disturbance. The important aspect of our theory concerns the primary source of energy for large-scale interplanetary transients. This we identify with the mechanism that accelerates high-speed streams and not with the impulsive release of magnetic free energy in the chromosphere and the corona.

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1. Rust, D. M. *Science* **216**, 939–946 (1982).
2. Lanzerotti, L. J. *Solar System Plasma Physics* Vol. 3 (North-Holland, Amsterdam, 1979).
3. Gapper, G. R., Hewish, A., Purvis, A. & Duffett-Smith, P. J. *Nature* **296**, 633–636 (1982).
4. Tappin, S. J., Hewish, A. & Gapper, G. R. *Planet. Space Sci.* **31**, 1171–1176 (1983).
5. Ananthakrishnan, S., Coles, W. A. & Kaufmann, J. J. *J. geophys. Res.* **85**, 6025–6030 (1980).
6. Tappin, S. J., Hewish, A. & Gapper, G. R. *Planet. Space Sci.* **32**, 1273–1281 (1984).
7. King, J. H. *Interplanetary Medium Data Book* Suppl. 2 (National Space Science Data Center, Goddard, 1983).
8. Sanahuja, B., Domingo, V., Wenzel, K. P., Joselyn, J. A. & Keppler, E. *Sol. Phys.* **84**, 321–337 (1983).
9. Sheeley, N. R. Jr. & Harvey, J. W. *Sol. Phys.* **70**, 237–249 (1981).
10. Wu, S. T., Han, S. M. & Dryer, M. *Planet. Space Sci.* **27**, 255–264 (1979).
11. Pizzo, V. *J. geophys. Res.* **83**, 5563–5572 (1978).
12. Joselyn, J. A. & McIntosh, R. S. *J. geophys. Res.* **86**, 4555–4564 (1981).
13. Cane, H. V., Stone, R. G., Fainberg, J., Steinberg, J. L. & Hoang, S. *Sol. Phys.* **78**, 187–198 (1982).
14. Cane, H. V. & Stone, R. G. *Astrophys. J.* **282**, 339–344 (1984).
15. Borrini, G., Gosling, J. T., Barnes, S. J. & Feldman, W. C. *J. geophys. Res.* **87**, 4365–4373 (1982).
16. Wu, S. T., Dryer, M. & Han, S. M. *Sol. Phys.* **84**, 395–409 (1983).

Fractal growth of viscous fingers: quantitative characterization of a fluid instability phenomenon

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What happens when one attempts to push water through a fluid of higher viscosity? Under appropriate experimental conditions, the water breaks through in the form of highly branched patterns called viscous fingers. Water was used to push a more viscous but miscible, non-newtonian fluid in a Hele-Shaw cell. The resulting viscous finger instability was found to be a fractal growth phenomenon. Reproducible values of the fractal dimension d_f were found and were interpreted using a modification of the diffusion limited aggregation model.

WHEN one attempts to use a low viscosity fluid (like water) to push high viscosity fluid (like oil), there arises a fluid instability phenomenon termed the 'viscous fingering instability' in which the low viscosity fluid forms characteristic fingers extending well into the high viscosity fluid[1-4]. In addition to its obvious interest in enhanced oil recovery by water flooding, and other practical applications, this phenomenon is of considerable basic interest and is not understood at present. It may be studied in detail in the laboratory using a Hele-Shaw cell, which is a pair of transparent plates separated by typically 0.5 mm. The space between the plates is filled with high viscosity fluid and the low viscosity fluid is injected. Normally, the interfacial tension between the two fluids is high and the low viscosity fluid forms non-fractal patterns that resemble the fingers of a glove[1,2]. Fractal phenomena are found to be associated with a range of random systems[5-8]. For this reason it is of interest to inquire whether or not there exist experimental conditions under which viscous fingers obey a fractal symmetry.

Experiments

Fractal structures in nature typically occur when randomizing effects dominate over stabilizing effects. Hence to find a fractal structure we must eliminate stabilizing effects so that the randomness can dominate a structure. To this end we shall minimize the role of interfacial tension[1,2] as well as intermolecular diffusion[3]. Therefore, we chose a viscous fluid with zero interfacial tension against water, an aqueous polymer solution of high relative molecular mass polysaccharide for which the mixing time is very slow compared with the time of the experiment.

Since the viscosity difference is the driving force for the instability, a high viscosity is desirable. Hence we use a non-newtonian fluid: the low-shear regions have high viscosity while the shear thinning character of the fluid makes it easy to pump it through the narrow gap of the Hele-Shaw cell without causing

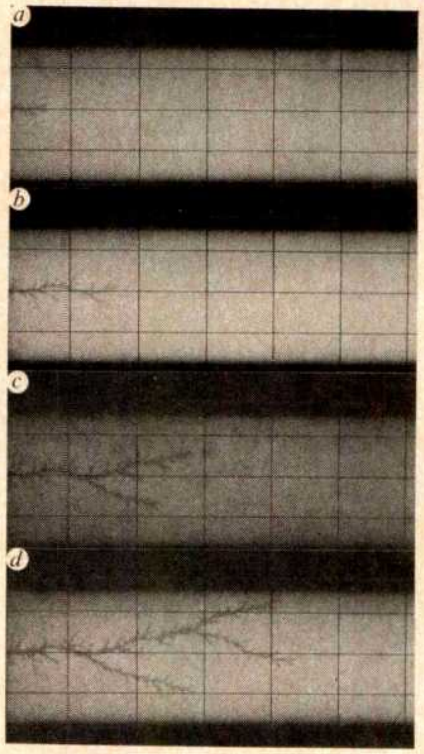


Fig. 1 Typical viscous finger created by water advancing into a Hele-Shaw cell filled with a non-newtonian fluid with zero interfacial tension (experimental conditions described in the text). The time interval between successive photographs is typically 5 s. The grid rectangles have dimensions 3 cm × 5 cm. The water, dyed with methylene blue, is injected under force from a single inlet on the left wall into an aqueous polymer solution whose viscosity varies between 10^3 and 10^4 cP depending on the water velocity.

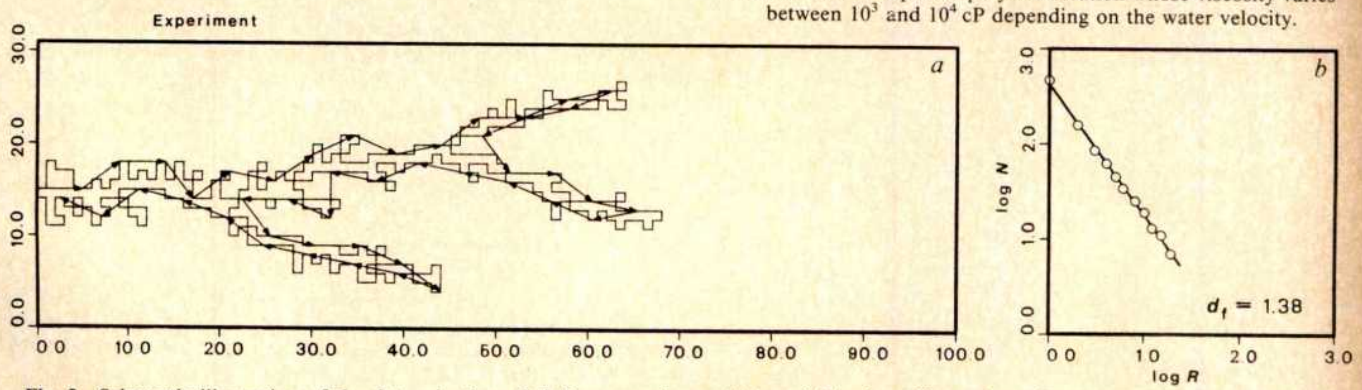


Fig. 2 Schematic illustration of the determination of d_f from experimental data of Fig. 1. *a*, Illustration of how the contour length of the digitized finger is measured with a ruler of length $R = 5$. *b*, The slope of the straight line gives $-d_f$, from which we find $d_f = 1.39$.

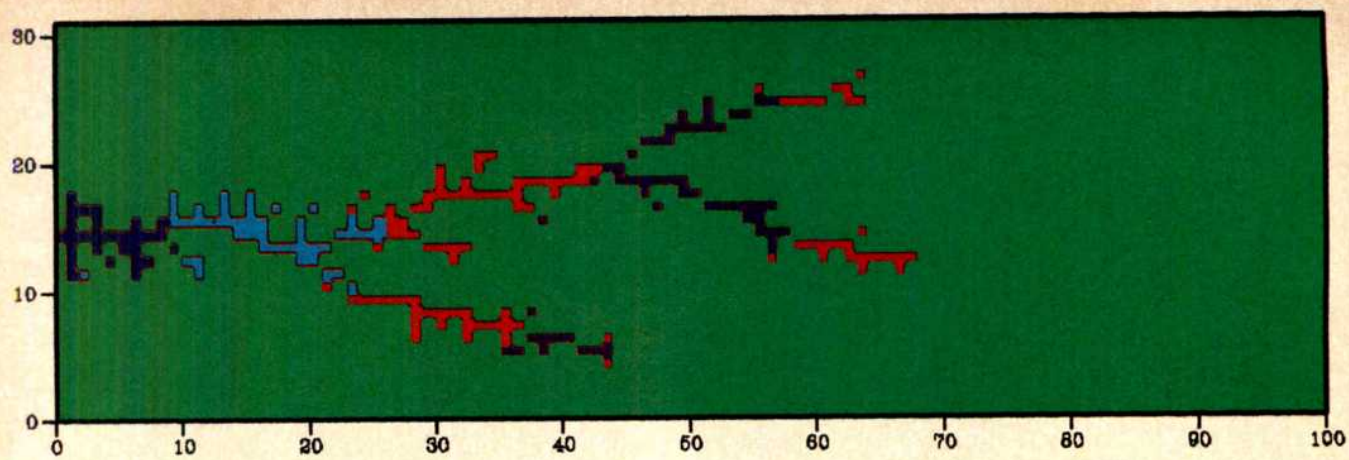
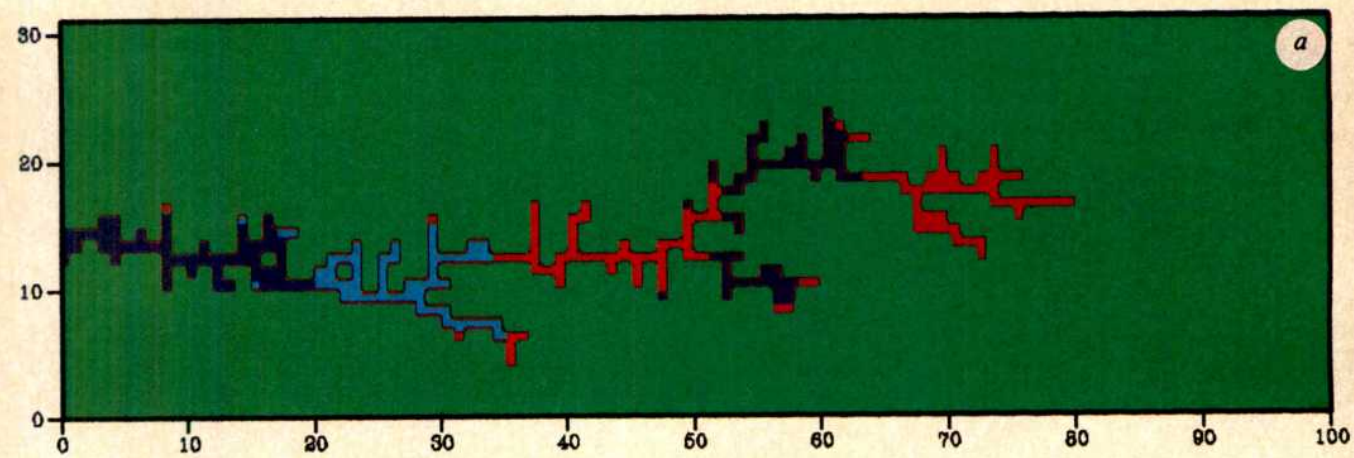


Fig. 3 Digitized representation of the viscous finger of Fig. 1. The colour coding is an indication of the time evolution of the instability, the order being dark blue, light blue, red, violet and pink. The colour coding is for comparison with the model calculations of Fig. 4.



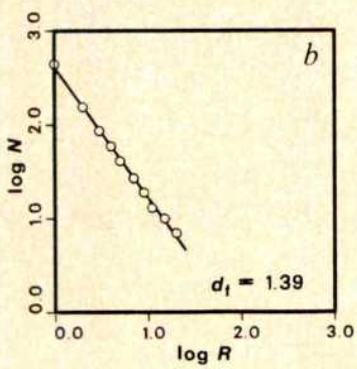


Fig. 4 Results of model calculation for the ratio $W_{HS}/W_f = 30$, corresponding to the experiments of Fig. 1. *a*, Colour coding indicates the time of arrival of the random walker, the order being dark blue, light blue, red, violet and pink; each colour represents 20% of total time. Comparison with Fig. 3 confirms the agreement between experiment and the model. *b*, The fractal dimension of the aggregate shown is 1.39, in agreement with the analysis of the experimental data of Fig. 2.

bending of the plates. In contrast, tests with newtonian fluids were limited to a viscosity of 100× that of water, and steady fine-structured fingering patterns were not observed.

We placed the polymer solution into a perpex Hele-Shaw cell[8] of thickness 0.5 mm, length 0.9 m and width 0.1 m. Water dyed with methylene blue was injected at one end at a variety of flow rates between 0.4 and 40 ml min^{-1}. We used three different polysaccharides and have varied the polymer concentration from 0.24 to 1.2 wt %. The viscosity of the polymer solution varies between 10^3 and 10^4 cP depending on the water velocity. We have also repeated measurements using a Hele-Shaw cell of thickness 1.0 mm. The resulting viscous finger patterns were random and highly branched (Fig. 1, *a*–*d*).

Analysis

Visual examination of these patterns reveals the striking presence of structure at many different length scales. Hence it seemed appropriate to attempt to analyse the structure of a viscous finger using the concept of self-similarity or fractals[5]. To this end, we first digitized the fingering patterns (Fig. 3) using a pixel size equal to the characteristic thickness of a viscous finger. We then analysed the patterns as follows. We measured the total exterior perimeter length using a 'ruler' of length $R = 1$, measured in units of a pixel. Then we repeated the same operation for a range of increasing R values up to $R = 20$ (Fig. 2*a*). The apparent length of any fractal, in units of R, decreases as R^{-d_f} (Fig. 2*b*)[5,9]. The d_f of the external perimeter is the same as the d_f of the total mass if loops do not occur on all length scales[5]. We find the quantitative parameter, $d_f = 1.40 \pm 0.04$, which characterizes viscous fingers for a wide range of polysaccharides, polymer concentrations and water flow rates, suggesting that viscous fingers indeed have a reproducible and universal fractal dimension.

As a second method, we placed an imaginary box of edge L on each point of the finger. For each box, we counted the number of points (the total mass) inside the box. As L increases, this mass should scale as L^{d_f} if the finger has a fractal structure[10]. We find that Method 2 gives smaller values for d_f than predicted by Method 1. The difference is ~8 % and is explained by the fact that a large proportion of the finger points lie on the

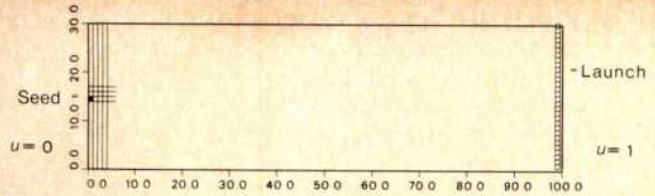


Fig. 5 The basic geometry of the generalized Witten-Sander model used to simulate viscous fingers. Random walkers are released from a randomly chosen point on the right wall, and 'die' if they strike the lateral or end walls.

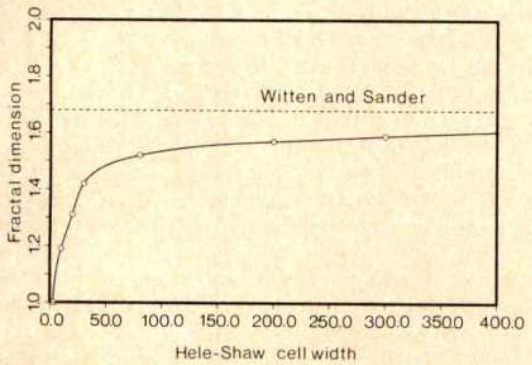


Fig. 6 Dependence on W_{HS} of the apparent value of the fractal dimension, d_f.

perimeter of a particular pattern and boxes centred at these points will cover much unoccupied area outside the structure. For the large-scale numerical simulations discussed below, the number of points on the perimeter is small compared with the total number of points; both methods give identical fractal dimensions.

Statistical model

The mean velocity, v, of a viscous fluid in a Hele-Shaw cell is given by the gradient of a potential $\phi = -(b^2/12\,\mu)\,P$, where b is the plate separation, μ is the shear viscosity and P is the fluid pressure[11]. For incompressible fluids, div $v = 0$; hence we obtain a Laplace equation for the potential distribution

$$\nabla^2\phi = 0 \qquad (1)$$

If, as in our case, the viscosity ratio between the two fluids is very large, we can approximate the potential in the less viscous fluid to be a constant ($\phi = \phi_0$). At the outlet, ϕ takes on a different value $\phi = \phi_1$.

With these boundary conditions, equation (1) is identical to the Laplace equation describing the probability $u(r, t)$ of a random walker to be at the point r at the time t[12,13]. This is similar to diffusion-limited aggregation (DLA)[13–16], so we propose the following quantitative model for viscous fingering. Place a seed particle at the origin (Fig. 5) and release a random walker from a randomly chosen point on the opposite wall. If the random walker hits the seed, it sticks, whereas if it hits any of the four walls, it 'dies' and a new walker is released. For the simulation, we have chosen our 'Hele-Shaw' cell width $W_{HS} = 30$ (in units of a lattice constant), as the experimental value of $x = W_{HS}/W_f$ is ~30, with W_f the characteristic size of a single finger. Formally, the probability $u(r, t)$ can be mapped onto the fluid potential $\phi(r, t)$ described above, $u(r, t) = (\phi - \phi_0)/(\phi_1 - \phi_0)$. Thus, $\phi - \phi_0$ in the finger corresponds to $u = 0$ in the aggregate, whereas $\phi = \phi_1$ at the outlet maps to $u = 1$ at the source of random walkers.

Two striking features of the actual viscous finger experiments are mirrored by our statistical model. The first of these is the tendency of a growing side branch to die out after a relatively short period of growth. The second is the fact that the entire viscous finger strongly avoids coming even moderately close to the lateral walls of the Hele-Shaw cell. Both effects are seen

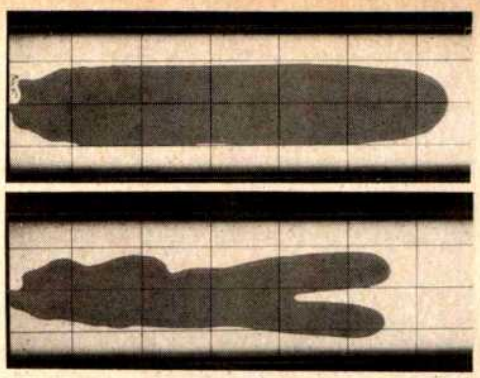


Fig. 7 A viscous finger created by water advancing into a Hele-Shaw cell filled with a newtonian fluid, oil. Two different capillarity numbers are used: *a*, $N_{ca} = 0.08$; *b*, $N_{ca} = 0.16$.

clearly in the model and arise from a common source, 'screening'[13–16]. This screening can be understood as follows. A random walk, statistically speaking, sticks to the tips of the growing aggregate so that the main trunk of the aggregate effectively shields the smaller side fingers. As the lateral walls absorb the random walkers, they act as 'ghost fingers': the viscous finger and the wall together form an essentially impenetrable deep fjord. This can be seen dramatically by the colour coding of Fig. 4, where the last random walkers to arrive, coloured pink, rarely reach the side fingers and never penetrate the deep fjord between the aggregate and the wall.

Calculating d_f as described above, we find $d_f = 1.41 \pm 0.05$ (see Fig. 5), a value remarkably close to the experimental value but considerably different from the value $d_f = 1.68 \pm 0.04$ for DLA[17]. Could this difference arise from the geometric constraint of the lateral walls? To test this possibility, we made additional simulations for a range of values of x. We find (Fig. 6) that the apparent value of d_f increases smoothly from $d_f \cong 1$ (for $x \cong 1$) to $d_f \cong 1.60$ (for $x \cong 10^3$). To check this prediction, we also performed experiments for a Hele-Shaw cell half as wide ($x = 15$) and find that $d_f = 1.12 \pm 0.04$, consistent with the predicted value of Fig. 6. What is the source of this apparent dependence of d_f on W_{HS}? At first sight, one might be tempted by an analogy with a growing polymer chain in the same confining geometry; it takes on the bulk value of d_f until it has grown sufficiently to sense the presence of the lateral walls. However, our problem is quite different, since the lateral walls strongly influence even the initial stages of finger growth because the incoming random walks are strongly influenced by the absorbing lateral and end walls. Hence the small fingers cannot capture enough mass to achieve the full DLA structure (this fact becomes especially clear if we consider very small values of W_{HS}). So while for the polymer problem d_f crosses over directly from its bulk value to its ultimate value of $d_f = 1.0$, for the Hele-Shaw fingering problem d_f never takes on its bulk value but instead takes on a smaller value that depends monotonically on W_{HS} (Fig. 6), crossing over ultimately to $d_f = 1$ when $R \gg W_{HS}$.

Discussion

We consider here the relation of the present experiments to previous work on viscous fingering. The classic Saffman–Taylor work did not produce ramified fractal structures. To obtain fine-structured instability patterns for an immiscible fluid system, the critical wavelength λ_c of a perturbation[2,18], defined as $\lambda_c = k[b^2/(12\,N_{ca})]^{1/2}$ has to be much smaller than W_{HS}. N_{ca} is the capillarity number, defined as $N_{ca} = \mu v/\sigma$ (σ is the interfacial tension) and corresponding to the ratio of viscous forces to capillarity forces: k is a dimensionless parameter that depends on the contact angle between the two fluids and the plates (wettability)[18]. We have also performed Saffman–Taylor type experiments with an immiscible newtonian oil–water system with varying flow velocity v, and Fig. 7 indicates that a change

in the flow condition from $N_{ca} = 0.08$ to 0.16 introduces a bifurcation into fingers when the finger thickness exceeds twice λ_c[19]. A continuation of this process would presumably create smaller and smaller fingers with a tree-like structure, but would require a further increase in the capillarity number or a decrease of the plate distance b. We cannot test the possibility that newtonian fluids with very low interfacial tension and negligible intermolecular diffusion but with a very high viscosity ratio, might give rise to similar fractal instability patterns, as our experiments were limited by the finite strength of the cell. By using water and a non-newtonian water-based fluid, we obtained a flow with an infinite capillarity number and a high viscosity ratio in regions where shear stresses are low. We believe both aspects are important and explain the difference from the Saffman–Taylor results.

Our present findings can be applied to the basic physics underlying petroleum science[4,20]. In the traditional '5-spot well configuration', four production wells are drilled at the corners of a large square and CO_2 (or a low viscosity fluid with a very low interfacial tension with oil) is then pumped into a fifth injection well in the centre. The CO_2 plays the role of the water in our model experiments, whereas the oil is analogous to the polymer solution. Large-scale viscous fingering phenomena not bounded by the walls of a Hele-Shaw cell occur and drastically reduce the efficiency of the oil recovery. The observed viscous fingers are quasi-two-dimensional as the thickness of the rock formation is typically several orders of magnitude less than the separation of the injection and production wells. It is therefore customary to assume that the patterns observed in the quasi-two-dimensional Hele-Shaw cells are relevant to the observed macroscopic phenomena. The similarity in the mathematical description of flow through porous media and the Hele-Shaw cell have been observed previously[1]. Thus, our experiments and model calculations strongly suggest that the viscous fingers observed in a 5-spot pattern are rich geometrical structures possessing a scale-invariant symmetry and quantitatively characterized by a fractal dimensionality.

Conclusion

There is no complete theory of miscible viscous fingering, perhaps because there was no quantitative parameter to characterize viscous fingers. Here, we find that viscous fingers are fractals and that their many different random structures have the identical value of the fractal dimensionality. This suggests that the physical basis of the viscous fingering instability is analogous to that of other second-order phase transitions that obey the same sort of scale invariant symmetry found here for viscous fingers[21]. Recent work supports our point of view: quasi-two-dimensional dielectric breakdown seems to exhibit the same sort of fractal structure that we find for the viscous fingering instability[22]. That these two breakdown phenomena should be related is plausible in light of the parallels in the equations describing them[23].

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Note added in proof: Since acceptance of this article, we have learned of independent work on viscous fingering, both from the experimental (ref. 24, and L. M. Sander and E. Ben-Jacob, personal communication) and theoretical[25,26] points of view.

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1. Saffman, P. G. & Taylor, G. I. *Proc. R. Soc.* **A245**, 312–329 (1958).
2. Chuoke, R. L., Van Meurs, P. & Van der Poel, C. *J. petrol. Tech.* **11**, 64–70 (1959).
3. Stalkup, F. I. *Miscible Displacements* (Soc. of Petroleum Eng., AIME, N.Y., 1983).
4. Hele Shaw, J. S. S. *Nature* **58**, 34–36 (1898).
5. Mandelbrot, B. B. *The Fractal Geometry of Nature* (Freeman, San Francisco, 1982).
6. Brady, R. M. & Ball, R. C. *Nature* **309**, 225–229 (1984).
7. Avnir, D., Farin, D. & Pfeiffer, P. *Nature* **308**, 261–263 (1984).
8. Matsushita, M., Sano, M., Hayakawa, Y., Honjo, H. & Sawada, Y. *Phys. Rev. Lett.* **53**, 286–289 (1984).
9. Powles, J. G. & Quirke, N. *Phys. Rev. Lett.* **52**, 1571–1574 (1984).
10. Forrest, S. L. & Witten, T. A. *J. Phys.* **A12**, L109–112 (1979).
11. Lamb, H. *Hydrodynamics* (Cambridge University Press, London, 1932).
12. Chandrasekhar, S. *Rev. mod. Phys.* **15**, 1–89 (1943).
13. Witten, T. A. & Sander, L. M. *Phys. Rev. Lett.* **47**, 1499–1501 (1981).
14. Witten, T. A. & Sander, L. M. *Phys. Rev.* **B27**, 5685–5697 (1983).
15. Herrmann, H. J. *Phys. Rep.* (in the press).
16. Stanley, H. E. *Fractals in Statistical Physics* (Oxford University Press, New York, 1985).
17. Meakin, P. *Phys. Rev.* **A27**, 1495–1507 (1983).
18. Peters, E. J. & Flock, D. L. *J. Soc. petrol. Engng* **21**, 249–258 (1981).
19. Paterson, L. *J. Fluid Mech.* **113**, 513–529 (1981).
20. Todd, M. R. & Longstaff, W. J. *J. petrol. Tech.* **253**, 874–882 (1972).
21. Stanley, H. E. *Introduction to Phase Transitions and Critical Phenomena* (Oxford University Press, 1971).
22. Niemeyer, L., Pietronero, L. & Wiesmann, H. J. *Phys. Rev. Lett.* **52**, 1033–1036 (1984).
23. Paterson, L. *Phys. Rev. Lett.* **52**, 1621–1624 (1984).
24. Maher, J. V. *Phys. Rev. Lett.* (submitted).
25. Tang, C. *Phys. Rev. A* (submitted).
26. Kadanoff, L. P. *J. stat. Phys.* (submitted).

LETTERS TO NATURE

A very bright water vapour maser source in the galaxy NGC3079

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The 6_{16}–5_{23} transition of water vapour at 22.235080 GHz has been found in our Galaxy to be associated with regions of young star formation[1,2]. H_2O maser emission was detected initially in an external galaxy by Churchwell *et al.*[3]. To extend the range of detected properties of 'star burst' galaxies (galaxies showing an enhanced star formation from their optical spectra) we undertook an initial survey for H_2O maser emission in this type of galaxy, but with negative results. Here we report on the subsequent extension of this survey to spiral galaxies displaying continuum radio emission, which is also an indication of star formation activity. We have detected the most luminous H_2O maser source yet reported, ~500 L_0, in the galaxy NGC3079. OH absorption was also detected in this galaxy and we suggest that maser amplification of the nuclear radio source causes the observable H_2O emission.

The 30 galaxies for the survey reported here were selected from lists[4,5] of bright spiral galaxies containing radio sources having both compact and extended components. The observations were carried out in March, April and May 1984 using the 37-m radio telescope of Haystack Observatory, equipped with a maser receiver and a 1,024 channel digital auto-correlator. The system temperature is ~90 K and the bandwidths 20 and 40 MHz, providing resolutions of 0.8 and 3.2 km s^{-1}, respectively, at the rest frequency of 22.235080 GHz. Each source was observed in the total power mode for 3 h; the antenna beam is 90 arc s and covers only the central part of each galaxy.

In Fig. 1 is a spectrum for NGC3079 showing a strong central water vapour feature of ~5 Jy in size and having a width of ~10 km s^{-1}; in addition, there are weaker emission features of <1 Jy extending out to velocities of ±80 km s^{-1} from the central feature. The strongest feature in NGC3079 is blueshifted by ~200 km s^{-1} from the systemic velocity of the galaxy of 1,171 km s^{-1} (ref. 6). In Fig. 2 is a spectrum (taken on the NRAO 300-ft telescope in May 1984) of the absorption by the OH radical at 1667.358 MHz in the radio continuum of NGC3079 (see also B. Turner, I. Kazes and L. J. Rickard, personal communication). This spectrum shows a deep absorption feature ($N_{OH}/T_{EX} = 7.6 \times 10^{15}$ cm^{-2} K^{-1}) at lower velocities than the systemic velocity of the galaxy. The velocity of the H_2O maser emission is indicated lying on the blueshifted edge of the OH absorption, implying that the H_2O maser source is foreground

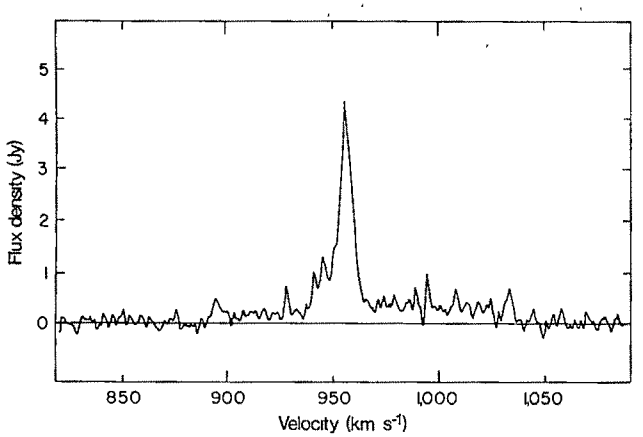


Fig. 1 A spectrum of NGC3079. The rest frequency is 22.235080 GHz and the resolution is 1.3 km s^{-1}. Velocity is given by $c\Delta\lambda/\lambda_0$, where c is the velocity of light and λ, the wavelength.

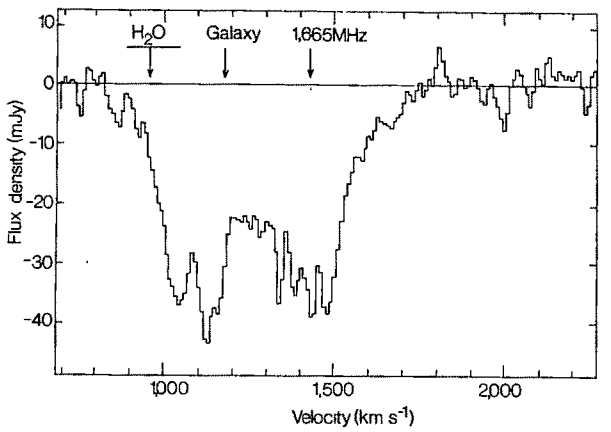


Fig. 2 An OH spectrum of NGC3079 taken on the NRAO 300 ft antenna in Greenbank, West Virginia. The rest frequency used is 1,667.358 MHz. Also indicated on the plot are the velocities of the H_2O maser source and the 1,665 MHz absorption feature. The 1,665 and 1,667-MHz absorption features are slightly blended. The galaxy velocity is also indicated. This absorption feature has been independently detected by Turner, Kazes and Rickard (personal communication).

to the radio source. It is likely to be close to the nucleus and part of an outflow of gas from the galaxy nucleus.

The H_2O spectrum of NGC4258 is given in Fig. 3 and shows a significant change from that given by Claussen, Heiligman and Lo[7] for January 1983 in that a feature at 455 km s^{-1} has risen to a level of ~10 Jy. Spectra taken in August 1984 (G. Garay, J. M. Moran and A.D.H., unpublished results) show this feature to have declined to ~5 Jy. As the weak feature at ~490 km s^{-1} is approximately the same amplitude of ~1.5 Jy in the January 1983 spectrum as in April 1984, it may be constant, with the difference in shape being attributable possibly to the poorer signal/noise ratio of the later spectrum. The linear polarization of the brightest velocity features in the maser sources in NGC3079 and NGC4258 was measured (G. Garay, J. M. Moran and A.D.H., unpublished results) using the Haystack antenna and found to be <6% for NGC3079 and <2% for NGC4258. The non-detections of galaxies are listed in Table 1 together with their upper limits.

The maser source in NGC3079 is the most luminous maser source yet detected having a peak isotropic luminosity in June 1984 of ~500 L_0, assuming a distance to NGC3079 of 16.5 Mpc ($H_0 = 75$ km s^{-1} Mpc^{-1}). NGC3079 and the previously detected galaxies NGC4258 (130 L_0, this observation) NGC1068[7] (350 L_0), NGC4945[8] (240 L_0)[9] and the Circinus Galaxy[10] (37 L_0), form a class of galaxies that contain highly luminous water vapour maser emission. For comparison, in our own

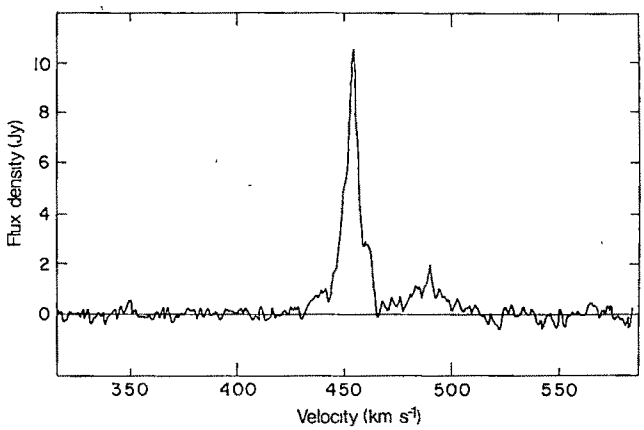


Fig. 3 A spectrum of NGC4258. The resolution and rest frequency are as in Fig. 1.

Table 1 Sources in which H_2O was not detected

Source	S_{lim} (3×r.m.s) (Jy)	Velocity (km s^{-1})
NGC520	0.5	2,100
NGC660	0.4	856
NGC891	0.4	72
NGC972	0.7	1,555
NGC1068	1.0	1,135
NGC1073	0 4	1,212
NGC1087	0.3	1,824
NGC1569	0 4	−42
NGC2146	0.5	812
NGC2276	0.6	2,391
NGC2903	1.0	644
NGC3034	0.7	186
NGC3628	0.4	842
NGC3690	0.12	3,100
NGC3310	0.4	1,026
NGC3504	0 5	1,526
NGC3556	0.4	697
NGC4051	1.2	658
NGC4102	1.1	897
NGC4151	1.1	457
NGC4157	0.5	771
NGC4303	1.5	1,671
NGC4321	0.7	1,617
NGC4565	0.5	1,183
NGC4656	1 1	755
NGC4631	0 6	626
NGC4490	0.5	570
NGC5055	0.6	520
NGC6946	0 5	80
NGC7331	0.4	950
NGC7714/5	0.7	2,833

Galaxy the strongest source known, W49N, has a luminosity of <1 L_0 and all other extragalactic water vapour masers detected thus far are <L_0.

One interpretation[7] for the high luminosities of the H_2O maser sources in these galaxies is that they could indicate a burst of star formation of 10^5–10^6 stars assuming that the maser emission is the sum of a number of individual sources and considering that the H_2O luminosity of an average star formation region in our galaxy is 10^{-3} L_0. This is similar to the burst of star formation of ~10^8 stars that would be predicted from a comparison of luminosities of the bright OH maser emission detected by Baan, Wood and Haschick[11] in the galaxy IC4553 (Arp 220).

An alternative explanation for these bright maser sources may lie in the supposition that we are seeing low-gain maser amplification of the background continuum radio source. A salient characteristic of these five galaxies is the presence of nuclear radio sources in all cases[5,8,9]. In the example of the OH megamaser IC4553, the emission is found to be exactly superimposed

on the nuclear radio source, which is resolved into three components; relatively low-grain maser amplification of the background source is suggested as being the mechanism for producing the intense OH-maser emission (ref. 12 and R. P. Norris, W.A.B, A.D.H., R. S. Booth and P. J. Diamond, in preparation), even though the masing region in front of the central component may be partially saturated. This indeed may also be the mechanism producing the intense H_2O emission in the above mentioned galaxies. Recent Very Large Array observations with a resolution of 2.5 arc s indicate that the brightest feature in NGC3079 is superimposed on the compact nuclear radio source. Furthermore M. J. Claussen *et al.* (personal communication) report that the strong maser feature in NGC1068 is coincident with one of the three radio-continuum knots in the nucleus of the galaxy and that the H_2O features in NGC4258 are coincident with the nuclear radio source in this galaxy. These positional coincidences may be characteristic of the emission mechanism responsible for the H_2O lines. The narrower H_2O velocity features, $\sim 10\,km\,s^{-1}$ for the main features seen in these galaxies, as compared with 108 $km\,s^{-1}$ in IC4553, would indicate higher-gain amplification by fewer H_2O masing regions than in the OH example and a smaller size for the H_2O sources. The brightness temperature of the H_2O features in NGC3079 will determine whether the amplification hypothesis is applicable in this case. However, a molecular region with an inverted H_2O population will amplify radiation from a background continuum source until the maser reaches saturation and is observable; without the background source the maser may be weaker or not observable.

Maser amplification of the background continuum would not eliminate the necessity for highly luminous pump sources for the masing regions and it is possible to postulate that bursts of star formation supply the energy for these maser sources. All the powerful extragalactic masers are associated with galaxies having both a high infrared luminosity, which is thought to indicate a large number of hot massive stars enshrouded by dust and signs of nuclear activity. Galactic H_2O maser sources are thought to be pumped collisionally[13], but as these extragalactic amplifying masers could be only partially saturated, the pump source may be radiative requiring only a modest burst of star formation ($<10^3$ stars) Pump models as suggested by Strelnitzkij[14] could present further alternatives to explain the luminous maser emission.

It also seems pertinent that three of the five luminous masing galaxies are edge-on galaxies (NGC1068 and the Circinus galaxy being the two exceptions), which raises the molecular column density and thus the probability of having H_2O masing regions on the line of sight to the nuclear radio source. The presence of OH absorption in two of the galaxies (NGC3079 (see Fig. 3 and B. Turner, I. Kazes and L. J. Rickard, personal communication) and NGC4945; ref. 15) indicates the presence along the line of sight of dense molecular clouds. The relatively large number of non-detections of strong H_2O maser sources in galaxies having radio continua would imply that the presence of a nuclear radio source is a necessary, but not sufficient, condition for this maser emission. Thus, it seems plausible that these superluminous extragalactic maser sources may simply comprise a number of individual masing regions which are foreground to the nuclear radio source.

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1 Lo, K Y, Burke, B F & Haschick, A D *Astrophys J* **202**, 81 (1975)
2 Genzel, R & Downes, D *Astr Astrophys Suppl* **30**, 145–168 (1977)
3 Churchwell, E *et al Astr Astrophys* **54**, 969 (1977)
4 Condon, J J *Astrophys J Suppl.* **53**, 459–495 (1983).
5 Israel, F. P & van der Hulst, J, M *Astr J* **88**, 1739–1748 (1983)
6 Nilson, P *Uppsala Astr Obs Anum* 6 (UGCG), 154 (1973)
7 Claussen, M J, Heiligman, G M & Lo, K Y *Nature* **310**, 298–300 (1984)
8 Dos Santos, P M & Lepine, J R D *Nature* **278**, 34–35 (1979)
9 Moorwood, A F M & Glass, I S *Astr Astrophys* **135**, 281–288 (1984)
10 Gardner, F F & Whiteoak, J B *Mon Not R astr Soc* **201**, 13P–15P (1982)
11 Baan, W A, Wood, P A D & Haschick, A D *Astrophys J Lett* **260**, L49–L52 (1982)
12 Baan, W A & Haschick A D *Astrophys. J* **279**, 541–549 (1984)
13 Downes, D in *Birth and Infancy of Stars* (eds Lucas, R & Omont, A) (in the press).
14 Strelnitzkij, V S *Mon Not R astr Soc* **207**, 339–354 (1984)
15 Whiteoak J B & Gardner, F F *Astrophys J* **195**, L81 (1974)

Periodicity in the BL Lac object OJ287

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Short-term variations in luminosity provide the best upper limits for the sizes of active galactic nuclei. Periodicity provides further information on the geometrical, dynamical and physical models of these regions. The best candidates for the study of such variations are the so-called blazars (optically violently variable (OVV) objects and BL Lac objects). Our photometric observations of OJ287 reveal periodic variability in timescales of a few minutes. We conclude that our results are compatible with the presence of 'hotspots' in a thick accretion disk spiraling into a Kerr's supermassive black hole and that the central source is seen nearly pole-on.

The highly variable nature of OJ287 at visible, radio and infrared wavelengths is well established[1,2]. However, the possibility of periodic light changes in this object has created some controversy[3–5]. Recent observations[6] at radio wavelengths (22 and 37 GHz) seem to indicate that OJ287 could vary regularly with a periodicity of 15.7 min. We have undertaken an observational program to search for regular time variations in the light of blazars, looking particularly at OJ287 as a promising candidate. The light curve of OJ287 has been observed with a 1-s time resolution, during eight night runs collected between January and March 1983, with two additional runs in February 1984. Table 1 summarizes the dates and duration of the observations. The observations were carried out using a high-speed two-channel photometer, developed by Nather[7], attached to the 1-m telescope at Tonantzintla Observatory. To maximize the photon counting rate, the observations were carried out without filter, using the full spectral response of the photomultiplier (RCA 8850) which yields an instrumental effective wavelength similar to that of Johnson's B band. This two-channel photometer allows simultaneous observations to be made of comparison sources, so enabling fluctuations in atmospheric transmission and extinction to be removed from the observed light curves. From photometry done through several apertures, we find that 99% of the light was contained within 15 arc s. To eliminate instrumental modulation, we chose a 29-arc s diaphragm. We applied the same observational and reduction techniques to stellar objects to determine the instrumental effects;

Table 1 Observational data

Date (UT)		Duration (s)
17.2722	January 1983	3,200
17.3104	January 1983	3,200
16.0674	February 1983	5,000
17.2681	February 1983	3,700
18 2556	February 1983	4,900
10.1146	March 1983	12,700
11.0847	March 1983	15,500
12.2201	March 1983	2,300
25.2688	February 1984	8,400
26.1257	February 1984	13,100

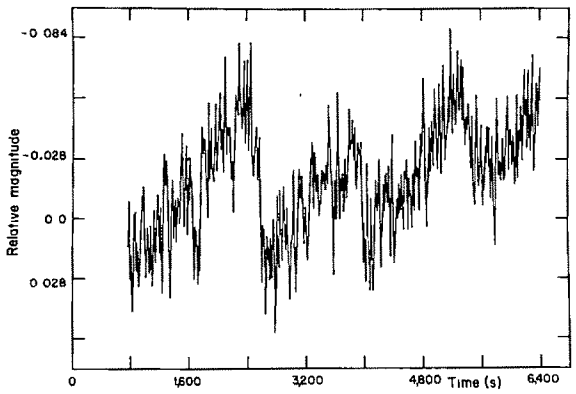


Fig. 1 Observed light curve for the night of 10 March 1983. $t=0$ corresponds to 2h 51.7 min (UT).

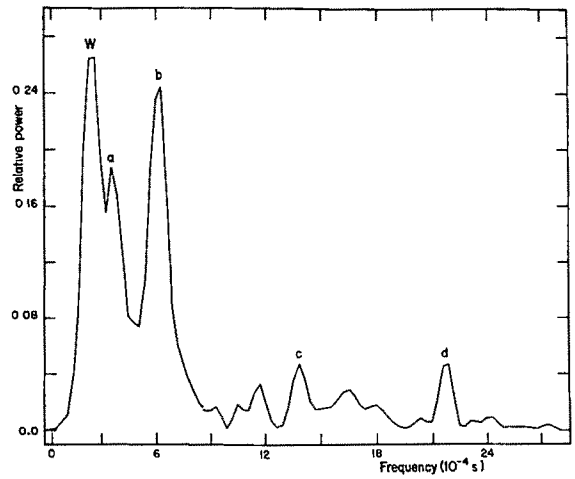


Fig. 2 Power spectrum derived from Fourier analysis with the Deeming algorithm for unevenly spaced points, applied to the 1983 data. Feature W corresponds to the observational time window. Features a, b, c and d are produced by the intrinsic shape of the light curve of OJ287 The corresponding periods are listed in Table 2

none were found that affected our conclusion regarding the periodicity of OJ287.

A typical corrected light curve of OJ287 during the 1983 active period is shown in Fig. 1. The periodicity of the light variations indicates a period of 23 min. Furthermore, a fast Fourier transform analysis of the time series has been performed on the individual runs and on all the observations carried out in 1983. For the latter, the Deeming[8] algorithm was used on zeroed and edge-smoothed data. In every case, we find significant power at the frequencies corresponding to the periods listed in Table 2 (see also Fig. 2). Our analysis implies that the detected light variations are coherent over at least the two months in 1983 during which the observations were carried out. The active period of OJ287 could have lasted a year, or be present again at the beginning of 1984, when the observations were repeated and the same results obtained. Note that the most evident frequency present in our data set is roughly double the frequency detected during the 1972 active period of OJ287.

From the Fourier analysis of individual nights, a little power at the fundamental frequency of the gear's periodicity was seen, corresponding to a period of 138 s The amplitude for the 23-min period, the most prominent one during the observation, was 0.030 ± 0.005 m. From the apparent magnitude of the object ($m_v\sim12$) at those times and a redshift $z=0.301$ (ref. 9), we obtain 4×10^{45} erg s^{-1} for the visible luminosity of the modulated component.

Note that at the epoch of our observations (1983–84), OJ287 was at maximum brightness ($m_v\sim12$), which was also the case 10 years ago when Visvanathan and Elliot[3] reported light variations with a 40-min period, which were confirmed a year later by Frohlich *et al.*[4]. On careful examination of the light curve in ref. 3, however, a period of ~20 min cannot be excluded.

One way in which short-period variations could be produced involves the pulsational modes of self-gravitating accretion disks around supermassive black holes[10]. Grauer[11] suggested that quasiperiodic optical variations of the OVV 4C29.45 over time scales ~20 min could be interpreted in terms of this idea. However, this model has to be worked out more carefully as the driving force behind those oscillations has not been analysed, nor is there any obvious reason to pick out a specific annulus of the disk that dominates the pulsations. A more sensible way of explaining short-period regular variations is to assume that the accretion disk contains one or various 'hotspots' which are spiralling into a black hole[12]. For a Schwarzschild hole, thin accretion disks have their innermost stable orbit at $6GM/c$, and thick disks reduce this value at $4GM/c$ (ref. 13). The radius of the event horizon of a Kerr black hole of mass M and angular momentum parameter a is (P. J. Wiita, preprint)

$$r=1.48\times10^5(1+\sqrt{1-a^2})(M/M_\odot)\ \text{cm} \qquad (1)$$

In the case of an extreme Kerr black hole ($a=1$), both the innermost bound and stable orbits coincide at $r=GM/c$. Thus a periodic phenomenon could exist down to timescales of

$$P=2\pi r/c\approx3.1\times10^{-5}(M/M_\odot) \qquad (2)$$

This expression yields a mass for the central black hole of $3\times10^7M/M_\odot$ for a 23-min period and $6\times10^7M/M_\odot$ for the 40-min one. These masses lead to Eddington luminosities of 5×10^{45} and 1×10^{46} erg s^{-1} respectively. These numbers are too small to explain the object's bolometric luminosity (we recall that at maximum light, $L_v\sim10^{47}$ erg s^{-1}). However, thick accretion disk models allow for actual luminosities that exceed L_E (ref. 14). Furthermore, one must still consider the effects of directed relativistic outflow (beaming)[15]. For a bulk relativistic Lorentz factor γ, the observed luminosity is enhanced by a factor of γ^2 and the total mass-energy required (for a fixed efficiency in the comoving frame) is reduced by a factor γ^4.

We conclude that the model that can explain our observations is one in which 'hotspots' in a thick accretion disk are spiralling down to a Kerr's supermassive black hole and are being periodically occulted by the disk itself. To have a beaming effect and to see the hotspots at the innermost part of the source, we must be viewing it almost pole-on (within $\sim11°$ of the rotation axis of the disk according to Sikora[16]) and the Thompson scattering depth τ_T, should be <1, which is likely to be the case for highly polarized objects (P. J. Wiita, preprint). The conflict between the real and observed luminosities can be resolved if an apparent luminosity of $\sim10\ L_E$ is produced by thick disk accretion coupled with bulk relativistic motions with $\gamma\sim2$.

Table 2 Observed periods

Fast Fourier transform (s)	Deeming (s)	Feature*
2,500†, 2,710	2,532	a
1,355	1,370	b
942‡	—	
—	721	c
455	459	d

* See Fig. 2. † See ref. 4. ‡ See ref 6

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1 O'Dell, S L, Puschell, J J, Stein, W A & Warner, J W *Astrophys J Suppl.* **38**, 267–286 (1978)
2 Andrew, B H, Harvey, G A & Medd, W J *Astrophys Lett* **9**, 151–154 (1971)
3 Visvanathan, N & Elliot, J L *Astrophys J* **179**, 721–730 (1973)
4 Frohlich, A, Goldsmith, S & Weistrop, D *Mon Not R astr Soc* **168**, 417–425 (1974)
5 Kiplinger, A L *Astrophys J Lett* **191**, L109–L110 (1974)

6 Valtaoja, E *et al Nature* **314**, 148-149 (1985)
7 Nather, R. E *Vistas Astr* **15**, 91-112 (1973).
8. Deeming, T J. *Astrophys Space Sci.* **36**, 137-158 (1975)
9. Miller, J S, French, H B & Hawley, S A *Pittsburgh Conf on BL Lac Objects* (ed A N Wolfe) 176-191 (University of Pittsburgh, 1978)
10. Vila, S C *Astrophys J* **234**, 636-640 (1979)
11 Grauer, A. D *Astrophys J* **277**, 77-81 (1984)
12. Sunyaev, R. A. *Soviet Astr* **16**, 941-943 (1973)
13 Paczynski, B & Wiita, P J. *Astr Astrophys* **88**, 23-31 (1980)
14 Abramowicz, M A. & Nobili, L. *Nature* **300**, 506-508 (1982)
15 Blandford, R. D & Rees, M J *Phys Scr* **17**, 265-274 (1978)
16 Sikora, M *Mon Not. R. astr Soc.* **196**, 257-268 (1981)

A 15.7-min periodicity in OJ287

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The BL Lac-type object OJ287, with a redshift $z = 0.306$ and a luminosity exceeding 10^{47} erg s^{-1} at maximum light, is among the brightest and most violently variable objects of its class. Rapid variations, with timescales of a few hours or less, have been reported over a wide range of wavelengths[1,2] and also in polarization[3], imposing strong constraints on the size and dynamics of the source[4,5]. The detection of periodic variations in OJ287, or in any other compact extragalactic source, would be even more important, as these could be related to rotation or orbital motion in the source. So far the only searches sensitive to periodic variations shorter than 1 h are the photometric observations of Visvanathan and Elliot[6], Frohlich *et al.*[7] and Kiplinger[8], in which a 40-min period in OJ287 was respectively found, confirmed and not detected. We report here the detection of a 15.7-min periodic flux variation in OJ287 in April 1981 at 37 GHz. The source has been subsequently monitored for 265 h at 22 and 37 GHz, as well as in optical photometry, and the same period has been seen in simultaneous observations. The peak–peak amplitude of the variation is 1–10% of the total intensity. Other periods may also exist.

In the Turku-Otaniemi quasar variability monitoring programme[9], 20 quasar-type objects are observed for a period of, in the mean, one week each month with the 13.7-m radio telescope of the Helsinki University of Technology at Metsähovi, Finland. Each source is observed for a period of 1-2 h. During the measurement, a computer printout for every single integration segment (usually 50 s) is provided for control of the observation. On inspecting the printouts, we found that OJ287 was behaving differently from other sources and that an approximately 15-min periodicity was at times clearly visible even among the large noise of single integrations[10]. Subsequently, we observed OJ287 on several nights to ascertain whether periodic variations are really present.

The radio observations were made between April 1981 and May 1983. OJ287 was monitored for a total 265 h during 47 separate observing runs. We have used different observational procedures and integration times to check that the periodicities are not artefacts of our data-collecting methods or the spacing of observations. (Details of the observational procedures are presented elsewhere[9].) A typical run lasted about 6 h, with one data point (~50 s of integration time) produced every 2 min.

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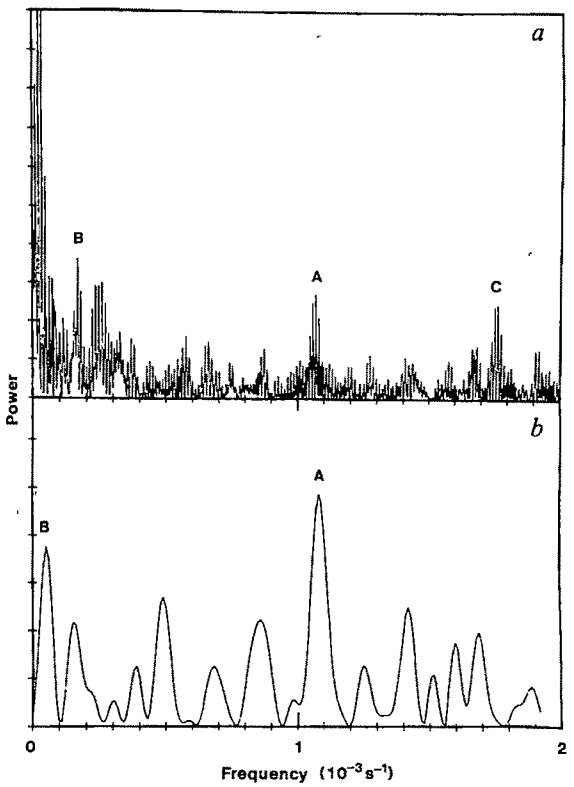


Fig. 1 *a*, Power spectrum of the 37-GHz observations with the Metsähovi radio telescope on 24 April 1981. A, the 15.7-min peak; B, peak caused by the data window. *b*, Combined power spectrum of the 22-GHz observations with the Metsähovi radio telescope on 5-7 March 1982. A, the 15.7-min peak; B, peaks caused by the data window and long-time changes in the signal level; C, peaks caused by instrumental effects.

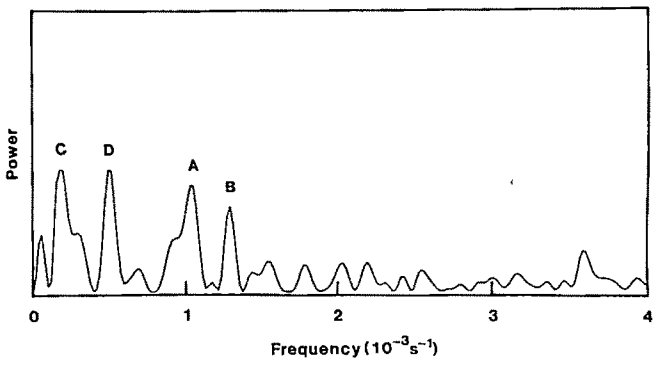


Fig. 2 Power spectrum of the 22-GHz observations with the NRAO 140-ft telescope on 23 May 1983. A, the 15.7-min peak; B, the 13.0-min peak; C, peak caused by the data window; D, a spurious peak, not seen in the Metsähovi power spectrum from the same day.

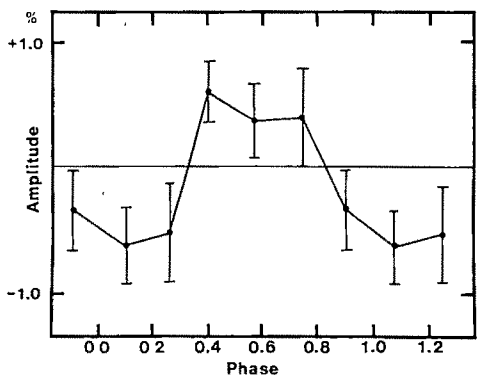


Fig. 3 The mean light curve of the 15.7-min variation from the NRAO observations. Error bars, $\pm 1\sigma$.

Thus, we had $n \sim 200$ points per run, with equal or unequal spacing (depending on the observing method) and some gaps attributable to calibration and optical depth measurements. The data for each run were then analysed by the Fourier technique of Deeming[11]. Because of the noise and data spacing, many peaks are apparent on the spectrum of a typical run. Among these peaks, one corresponding to a 15.7-min period is clearly visible on many nights and is shown in Fig. 1*a* for the run on 24 April 1981.

For a closer inspection of the observations, we have used the periodogram technique of Warner and Robinson[12], which is better suited for noisy data and non-sinusoidal variations. On nights where Fourier analysis indicates the presence of a clear 15.7-min variation, the periodogram technique helps to confirm the period, as well as giving the phase and the light curve of the variations.

On several occasions we were able to observe OJ287 on several consecutive nights. Although the long gaps between runs cause strong aliasing peaks, these can be identified by calculating the spectral window. If the 15.7-min variation is real, we would expect it to stand out more clearly in the combined data, whereas spurious peaks due to noise, unable to keep either phase or period from night to night, would be suppressed. Figure 1*b* shows the results of Fourier analysis for the combined runs of 5–7 March 1982 (595 observing points). Periodogram analysis gives a period of 943 s, in accordance with Fourier analysis. A comparison with similar data from combined runs, May 1981 onwards, indicates that the period may be constant to an accuracy of 1 s yr^{-1}.

To identify possible instrumental variations, we have made similar observations of a number of other sources and pure background noise, analysing these using the same techniques. Certain periodicities, for example, the peaks marked C in Fig. 1*b*, are caused by the radome and the variations in the electric current and are identified in most observing runs. Although no observing run is ever exactly repeatable and the periodicity could perhaps have been caused by some unknown mechanism, the most probable explanation is that the 15.7-min periodicity is intrinsic to OJ287, as we have not detected it in any of the other sources.

To ascertain whether the periodicity would be detectable with other instruments, simultaneous measurements were made on 26 February 1982 using the radio telescope at Metsähovi operating on 22 GHz, a double-beam chopping photometer using the 60-cm telescope of Metsähovi Observatory and using a similar photometer coupled to the 60-cm telescope at Turku Observatory, about 150 km west of Metsähovi. (The construction and operating principle of the photometers have been described by Piirola[13].) A standard observing procedure, with comparison stars measured at regular intervals, was followed. The measurements were made through the B filter. At the time of the observations, OJ287 was rather faint, with B (magnitude) = 14.6 mag, and the noise level of optical observations was rather high. The power spectra of all three runs were calculated and compared with each other and all three instruments showed a peak near the 15.7-min period: the radio telescope at 945 ± 15 s, the Metsähovi photometer at 980 ± 35 s and the Turku photometer at 935 ± 35 s, with the formal error limits derived from half-widths of the peaks.

To check for a coincidence, we further investigated all the peaks visible in each spectrum using the periodogram technique. In the case of the optical observations, the only periodicities found to have the same phase (within observational error) were those corresponding to the 15.7-min peak. In addition, the radio observations gave a similar light curve, but out-of-phase, and the amplitude of the variations seems to be the same at both wavelengths.

OJ287 was again observed with the 140-ft telescope of the National Radio Astronomy Observatory (NRAO) on 23 May 1983. The effective single integration times were 27 s and the total integration lasted 7.6 h, producing 330 observing points in all. Figure 2 shows the resulting power spectrum and Fig. 3 gives the mean light curve of the 15.7-min variation together with 2σ error bars. In addition to the 15.7-min power spectrum peak, strong peaks are visible at 33 min and 13 min. Both the 15.7-min peak and the 13.0-min peak were outstanding in the Metsähovi 37-GHz data (298 points over 6 h), also taken on 23 May 1983, but the 33-min peak was not observed.

Consequently, the 13-min periodic component, which has appeared on several occasions, has also attracted our attention. In addition, others[14] have observed a strong periodicity at 23 min between January and March 1983 in OJ287. Whether these periods are as durable as the 15.7-min period remains to be seen. Even in the case of the 15.7-min period, there are strong fluctuations in the amplitude, of $>15\%$ to $\sim 1\%$. An interesting parallel may be provided by the infrared observations of Wolstencroft *et al.*[2], where rapid, but presumably nonperiodic, variability was seen on only one night out of a total of 12, accompanied by a change in the shape of the infrared continuum. Simultaneous observations with different instruments are the only way to obtain definite proof for the existence of variations of this kind.

If only a single periodic component is confirmed, one may identify it with the rotation period of a single supermassive body[15–17]. However, if the evidence for many periodic components is sustained, then it is more probable that we are observing keplerian motion around a single dominant body with many different orbital radii, analogous to the modulation of the radio emission of Jupiter by its innermost moons. In any case, short periods such as 15.7 min indicate a relatively small mass for the central body ($\lesssim 10^7 M_\odot$) and a problem with the high luminosity of the quasar[4,5].

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1 Efanov, V A, Moiseev, I G, Nesterov, N S & Wright, A *Bull. Crimean astrophys Obs* **61**, 58–62 (1980)
2 Wolstencroft, R D, Gilmore, G & Williams, P M *Mon Not R. astr Soc.* **201**, 479–485 (1982).
3 Kulshrestha, A K, Joshi, U C & Deshpande, M R *Nature* **311**, 733–734 (1984)
4 Elliot, J L & Shapiro, S L *Astrophys J* **192**, L3–L6 (1974)
5 Abramowicz, M A & Nobili, L *Nature* **300**, 506–508 (1982)
6 Visvanathan, N & Elliot, J L *Astrophys J* **179**, 721–730 (1973)
7 Frohlich, A, Goldsmith, S & Weistrop, D *Mon Not. R. astr. Soc.* **168**, 417–425 (1974)
8 Kiplinger, A J *Astrophys J.* **191**, L109–L110 (1974)
9 Salonen, S. *et al Astr Astrophys Suppl* **51**, 47–62 (1983)
10 Lehto, H *et al. Turku University Observatory Informo* No 52 (1981)
11 Deeming, T J *Astrophys Space Sci* **36**, 137–158 (1974)
12 Warner, B & Robinson, E L *Mon Not R. astr Soc.* **159**, 101–111 (1972)
13 Piirola, V *Ann Acad. Sci. fennicae* A VI, No 418 (1975)
14 Carrasco, L., Dultzin-Hacyan, D & Cruz-Gonzalez, I *Nature* **314**, 146–148 (1985)
15 Morrison, P *Astrophys J* **157**, L73–L76 (1969).
16 Woltjer, L *Pont Acad Sci. Scripta Varia* **35**, 477–483 (1971)
17 Morrison, P & Cavaliere, A *Pont Acad Sci. Scripta Varia* **35**, 485–509 (1971)

Influences of precipitation changes and direct CO_2 effects on streamflow

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Increasing atmospheric carbon dioxide concentrations are expected[1] to cause major changes in the world's climate over the next 50–100 yr. The impact of such changes on water resources, through changing precipitation and evaporation, will, however, be complicated by the direct effects of increasing CO_2 on vegetation. In controlled environment experiments, higher CO_2 levels cause the stomata of plants to close down, decreasing their rate of transpiration and increasing their water use efficiency[2]. Reduced evapotranspiration would make more water available as runoff and could

tend to offset the effects of any CO_2-induced reductions in precipitation or enhance the effects of precipitation increases. We consider here, in a simple but revealing analysis, the relative sensitivity of runoff to these two processes, changes in precipitation and changes in evapotranspiration. We show that, for low runoff ratios, small changes in precipitation may cause large changes in runoff. The magnitude and direction of these changes is, however, strongly dependent on the magnitude of the direct CO_2 effect on plant evapotranspiration.

Although much has been published on the climatic effects of increasing CO_2, few studies have considered the impact on water resources. Revelle and Waggoner[3] used empirical relationships to study the effect on water supplies in the western United States of a 10% reduction in precipitation and a 2 °C warming. They suggested that there could be large reductions in river flow. Rind and Lebedeff[4] used the results of a general circulation model (GCM) to examine possible runoff changes in the United States in a high-CO_2 world. Their results differed from those of Revelle and Waggoner, partly because of differences between the model-generated changes in climate and the scenario assumed in the earlier work, and partly because GCM-generated runoff data cannot be directly equated with measured riverflow (see ref. 5). Neither of these studies considered the direct effects of CO_2 on vegetation. Following Aston[6] (who used a distributed deterministic process model to simulate the effects of changed stomatal resistance on streamflow), Idso and Brazel[7] added the direct CO_2 effects associated with a CO_2 doubling to Revelle and Waggoner's analysis. The 40–70% reductions in streamflow in the absence of these direct effects became 40–60% increases when they were included. Such increases are of the same size as those predicted by Aston for catchments in eastern Australia. But how representative are these results?

If non-evaporative losses are small the mean annual water balance of a river catchment is given by

$$R = P - E \tag{1}$$

where R is runoff (here synonymous with streamflow), P is precipitation and E is evapotranspiration. The runoff ratio, γ_0, is defined by

$$R_0 = \gamma_0 P_0 \tag{2}$$

where subscript zero is used to denote present-day values. To estimate changes in runoff due to changes in precipitation and evapotranspiration we denote the change in precipitation (from P_0 to P_1) due to a doubling of atmospheric CO_2 by

$$P_1 = \alpha P_0 \tag{3}$$

Changes in evapotranspiration will occur for two reasons, as a result of climatic change and through direct CO_2-induced changes in vegetation. If we use β to denote the fractional change in evapotranspiration

$$E_1 = \beta E_0 = \beta_1 \beta_2 \beta_3 E_0 \tag{4}$$

then β can be considered conceptually as the product of three factors, β_1 the change due to a change of climate, β_2 the change due to a change in the area of vegetated cover (due to either climatic change and/or direct effects of CO_2 on plant growth), and β_3 the direct CO_2 effect on evapotranspiration. For a doubling of CO_2, β_3 is given approximately (and conservatively) by

$$\beta_3 \simeq 1 - 0.3a \tag{5}$$

where a is the fractional vegetated area of the drainage basin (see ref. 7). Manipulation of the above equations yields

$$\frac{R_1}{R_0} = \frac{\alpha - (1-\gamma_0)\beta}{\gamma_0} \tag{6}$$

This result can be used to assess the relative sensitivity of runoff changes to changes in precipitation (α) and/or changes in evapotranspiration (β). In particular, direct CO_2 effects can be studied using equation (5). The relative sensitivity is given by

$$\left| \frac{\partial(R_1/R_0)}{\partial\alpha} \Big/ \frac{\partial(R_1/R_0)}{\partial\beta} \right| = \frac{1}{1-\gamma_0}$$

Thus, runoff is always more sensitive to precipitation changes than to evapotranspiration changes, particularly for higher values of γ_0. Individual sensitivities, however, are higher for small γ_0. $\partial(R_1/R_0)/\partial\alpha$ always exceeds one, so that precipitation changes have an amplified effect on runoff, particularly in arid regions where γ_0 is small. This is a well-known result (see, for example, ref. 8). $|[\partial(R_1/R_0)]/\partial\beta|$ exceeds one for $\gamma_0 < 0.5$, so evapotranspiration changes only have an amplified effect for small γ_0.

Figure 1 shows the change in runoff for various changes in annual mean precipitation in the presence of a maximum direct

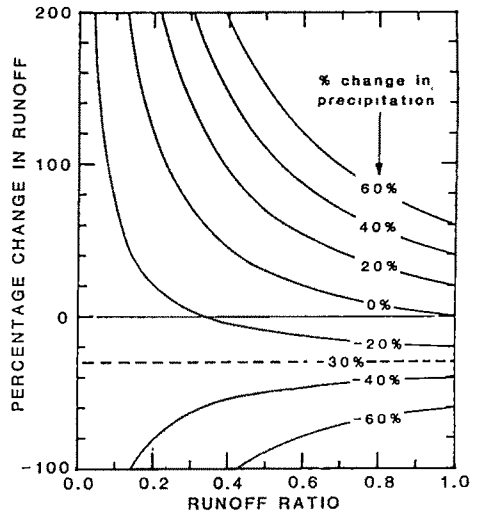


Fig. 1 Runoff changes due to changes in precipitation from a 60% reduction ($\alpha = 0.4$) to a 60% increase ($\alpha = 1.6$) assuming a maximum direct CO_2 effect on evapotranspiration ($a = 1$ in equation (5)) and no other changes in evapotranspiration ($\beta_1 = \beta_2 = 1$).

Table 1 Runoff ratios for some of the world's major rivers, listed approximately in order of mean annual discharge

River	Runoff ratio	River	Runoff ratio	River	Runoff ratio
Amazon	0.47	Parana	0.20	Niger	0.19
Congo	0.25	St Lawrence	0.33	Indus	0.40
Yangtze	0 50	Irrawaddy	0.60	Yukon	0.48
Brahmaputra	0.65	Ob	0.24	Nile	0.10
Ganges	0.42	Amur	0.32	São Francisco	0.15
Yenisey	0.42	McKenzie	0.40	Rhone	0.46
Mississippi	0.21	Columbia	0.51	Vistula	0.22
Orinoco	0.46	Zambezi	0 12	Volga	0.28
Lena	0.46	Danube	0.30	Murray	0.04

The values presented (based on ref 15) can only be taken as a general guide Data from different sources can vary widely because of difficulties in estimating natural flows and catchment area precipitation.

CO_2 effect ($a = 1$). For smaller runoff ratios (that is, more arid regions) any given change in precipitation has an increasingly large effect on runoff. When $\alpha = 0.7$ and $a = 1$, equation (6) reduces to $R_1 = 0.7\,R_0$, so that the runoff change is independent of the runoff ratio. For $0.7 < \alpha < 1.0$, runoff may increase or decrease depending on the value of γ_0. For $\alpha > 1.0$ runoff always increases (as the precipitation and evapotranspiration changes act in the same direction). The magnitude of the increase can be very large for small runoff ratios.

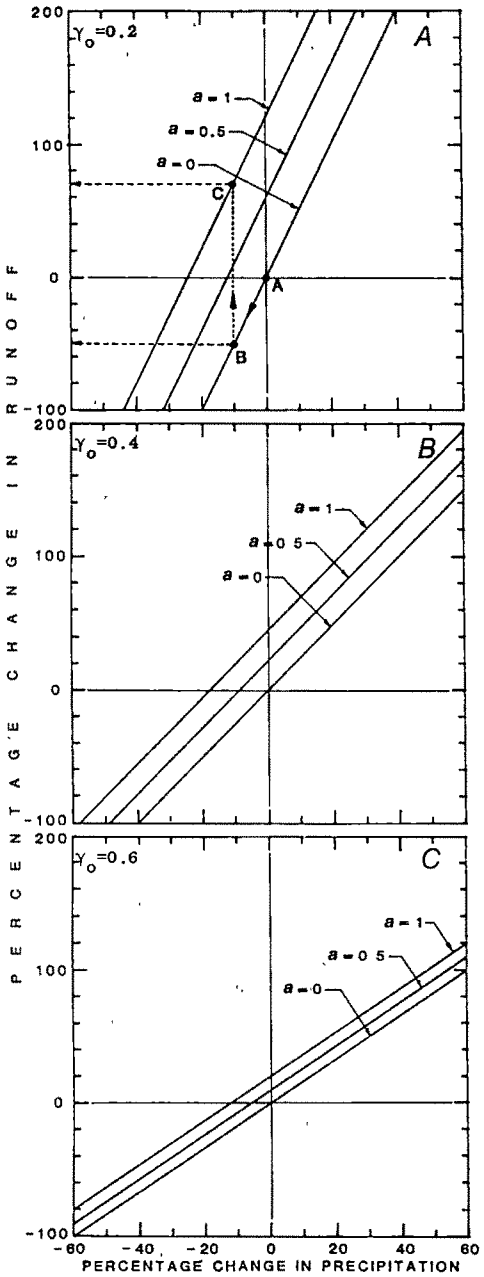


Fig. 2 Runoff changes due to precipitation changes for different evapotranspiration changes. $a = 0$ corresponds to no change in evapotranspiration, whereas $a = 1$ corresponds to a 30% reduction in evapotranspiration (the maximum direct CO_2 effect). The sensitivity to both precipitation change and evapotranspiration change increases for decreasing runoff ratio, γ_0. Three values of γ_0 are shown (*A*, $\gamma_0 = 0.2$; *B*, $\gamma_0 = 0.4$, *C*, $\gamma_0 = 0.6$) spanning the most common range of observed values *A* ($\gamma_0 = 0.2$) includes a specific illustration. The effects of precipitation change alone are determined by the $a = 0$ lines. The line AB, therefore, shows the effect of a 10% reduction in precipitation. The effects of evapotranspiration change are determined by paths parallel to the ordinate. Thus, BC corresponds to a range of evapotranspiration reduction from 0% (point B) to 30% (point C). Point C (and the lines $a = 1$ in general) corresponds to the maximum direct CO_2 effect, but the precise cause of the evapotranspiration change is unimportant.

Table 1 lists runoff ratios for some major rivers. Values range from 0.1 or less for rivers which flow through arid regions up to more than 0.6 for some tropical rivers. In temperate latitudes, runoff ratios around 0.4 are fairly typical. Equation (6) may not be directly applicable to rivers with the lowest runoff ratios in Table 1. Some of these obtain most of their water from relatively small, high precipitation headwater catchments and then flow through arid and largely unvegetated regions. The runoff ratios given in Table 1 are for the whole drainage basin and may, in these cases, fail to represent conditions in the hydrologically-active parts of the basin.

Figure 2 shows the sensitivity of the runoff-change–precipitation-change relationship to the direct effects of CO_2 for various values of the runoff ratio. The magnitude of the direct effect can be varied by changing the parameter a. When $a = 0$ the direct effect is zero, and when $a = 1$ the direct effect is a maximum. The effects of climate related changes in evapotranspiration can also be studied using Fig. 2 since the range $a = 0$ to 1 corresponds to a range of β values from 1.0 to 0.7, that is to a range of reductions in evapotranspiration from 0 to 30%. The slopes of the lines in Fig. 2 are greatest for smallest γ_0, showing that precipitation changes have maximum effect for small γ_0. Thus, for small γ_0 (as noted earlier), precipitation changes may show a considerably amplified effect in runoff.

As an example, for a catchment with present runoff ratio of 0.2, the effect of a 10% reduction in precipitation may range from a 50% reduction in runoff with no direct CO_2 effect, to a 70% increase in runoff with a maximum direct CO_2 effect. For higher runoff ratios the range of possible runoff changes is less (see Fig. 2). In both Aston's[6] study and the Idso and Brazel[7] study, runoff ratios were low, around 0.16 in the latter case. This low value explains why Idso and Brazel obtained such vastly different results from Revelle and Waggoner[3] when they incorporated the direct effects of CO_2. Table 1 shows that such low runoff ratios are unusual. Nevertheless, the direct CO_2 effect can still be substantial for more common runoff ratios. Idso and Brazel's case may also be unrepresentative of global conditions because they assumed precipitation reductions due to increasing CO_2 (following Revelle and Waggoner[3]). Climate model results all point to an increase in the intensity of the hydrological cycle and an overall (albeit slight) increase in global mean precipitation. There will, of course, be regionally and seasonally specific exceptions to this overall trend, but, in general, the effects of precipitation change and vegetation changes can be expected to reinforce each other. In some regions, therefore, we might expect very large increases in annual mean runoff, unless these are compensated by large climate-related increases in evapotranspiration.

The extent of this compensation is difficult to estimate because the magnitudes of climate-related changes in evapotranspiration due to increasing CO_2 are unknown. Over the land, evapotranspiration changes will result from several competing factors: warmer temperatures, changes in the length of the growing (evaporative) season, changes in wind speed, changes in cloudiness, and so on. Some of these factors may cause either increases or decreases in evapotranspiration. Although existing GCMs have recognized deficiencies in the way they model the hydrological cycle[9], they still provide the best available guide to the climatic effects of increasing CO_2. All GCMs show an increase in the intensity of the global hydrological cycle. The increases vary considerably from model to model and are larger for models which show greater global-mean warming. For a doubling of CO_2 the global-mean increases in precipitation and evaporation range between about 3% (ref. 10) and 11% (ref. 4). These increases in evaporation are much less than the maximum direct CO_2 transpiration effect. However, there may be appreciable regional and/or seasonal departures from the global mean (see ref. 4) so, at least in some regions, we might expect the climate effects to modify the direct effects significantly.

The above analysis ignores several important factors. For example, the seasonal distribution of precipitation may be very different from that of evapotranspiration, and direct CO_2 effects may be diminished if the major contribution to runoff is in the winter months[11]. Seasonal effects are, however, complex. For example, if E is defined as the residual of $P-R$ (equation (1)), then our results are independent of the seasonal distribution of precipitation. Direct CO_2 effects may also be reduced if increasing CO_2 causes an increase in leaf temperatures, in individual plant leaf areas or in the total vegetated area of a catchment[11,12]. More complex catchment models (such as the statistical models of Wright[13] and Jones[14], or deterministic models like that used by Aston[6]) are required to study these effects. Our study does, however, provide a basis for first estimates of annual mean runoff changes for any river whose runoff ratio is known, and allows one to identify regions most likely to experience large runoff changes due to increasing CO_2.

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1 NRC *Changing Climate* (National Academy, Washington DC, 1983)
2 Lemon, E R (ed) *CO_2 and Plants The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide.* A A A S Selected Symp. No 84 (Westview, Boulder, Colorado, 1983)
3 Revelle, R R & Waggoner, P E in *Changing Climate*, 419-432 (National Academy, Washington DC, 1983)
4 Rind, D & Lebedeff, S *Potential Climatic Impacts of Increasing Atmospheric CO_2 with Emphasis on Water Availability and Hydrology in the United States* (United States Environmental Protection Agency Report, 1984)
5 Nemeč, J in *Variations in the Global Water Budget* (eds Street-Perrott, A, Beran, M & Ratcliffe, R) 479-488 (Reidel, Dordrecht, 1983).
6 Aston, A R *J Hydrol.* **67**, 273-280 (1984)
7 Idso, S B & Brazel, A J *Nature* **312**, 51-53 (1984)
8 Schaake, J C Jr & Kaczmarek, Z in *Proc. World Climate Conf* W M O No 528, 290-312 (World Meteorological Organization, Geneva, 1979)
9 Dickinson, R E in *Geophys Monogr* No 29, Maurice Ewing Vol 5 (eds Hansen, J E & Takahashi, T) 58-72 (American Geophysical Union, Washington DC, 1984)
10 Manabe, S & Stouffer, R J *J geophys Res* **85**, 5529-5554 (1980)
11 Wigley, T M L, Briffa, K R & Jones, P. D *Nature* **312**, 102-103 (1984)
12 Morison, J I L & Gifford, R M *Aust J Pl Physiol.* **11**, 361-374 (1984)
13 Wright, C E *Synthesis of River Flows from Weather Data* (Tech Note No 26, Central Water Planning Unit, Reading, UK, 1978)
14 Jones, P D *J Climatol* **4**, 171-186 (1984)
15 Korzun, V I (ed) *World Water Balance and Water Resources of the Earth* (English transl, UNESCO, Paris, 1978)

Gypsum precipitation from cold brines in an anoxic basin in the eastern Mediterranean

Scientific staff of Cruise *Bannock* 1984-12*

In 1968 hot brines from which gypsum had precipitated were discovered in the Red Sea, in a divergent plate boundary setting with hydrothermal activity[1]. We now report the discovery of cold brines in an entirely different geodynamic situation, a convergent plate boundary in the eastern Mediterranean, where the heatflow is very low[2] and no hydrothermal activity occurs. Decimetric pure gypsum crystals precipitate from the cold brines, which occupy the bottom of a rimmed anoxic basin near the southern edge of the Mediterranean Ridge, close to the Sirte Abyssal Plain. Nucleation of the crystals does not occur at the surface, but from the deep brines, because the salinity of the water is normal down to the depth of −3,000 m. We propose the name 'Bacino Bannock' for this basin, after the Consiglio Nazionale delle Ricerche R/V *Bannock* from which the bottom-water brines were surveyed and sampled.

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Another anoxic basin containing highly saline bottom water was discovered last year in the western Strabo Trench, north o the Mediterranean Ridge, and named Tyro Basin[3]. Bacin Bannock differs from Tyro in that gypsum precipitation is onl recorded in the former.

Bacino Bannock was discovered using Seabeam and Seamar I during the *Robert D. Conrad* Cruise 25-06 (April 1984). I September 1984, *Bannock* Cruise 84-12 completed a narrow beam echosounder bathymetric survey of the basin, obtaine 10 cores and two dredges, and conducted three CTD (conduc tivity/temperature/depth) stations and one Nansen-bottl hydrocast. Navigation on both *Conrad* and *Bannock* was b Loran-C and transit satellites.

Bacino Bannock is an irregular depression (Fig. 1) about nautical miles (15 km) in diameter, within the irregular 'cobble stone topography' of the Mediterranean Ridge. A prominen bulge occupies the centre of the basin, surrounded by a moat-lik depression. The Bannock sampling programme was concen trated on, and to the west of, the central bulge, where th depression is deepest.

Sediment cores (Fig. 2) from 3,000 m and shallower contai the typical eastern Mediterranean pelagic stratigraphy o sapropels and tephras. However, bottom sediments collecte deeper than 3,200 m are black to dark grey and smell strongl of hydrogen sulphide. This anoxic environment contains stratigraphy of tephra layers, breccias, carbonate sands an graded sands and silts. All samples examined at sea were richl fossiliferous, with varying proportions of planktonic and dis placed benthic foraminifera (*Asterigerinata mamilla, A. planor bis, Asterigerina* sp., *Gavelinopsis lobatulus, Rosalina globularis* etc.), calcareous nannoplankton and siliceous plankton. Anoxi sediments are over 9 m (implying a sedimentation rate o 10 cm kyr^{-1} in our longest core) and range in age from Holocene probably to middle Pleistocene, as based on a combination o calcareous nannofossil biostratigraphy (*Gephyrocapsa oceanic* Zone) and planktonic foraminiferal palaeoecology.

A basal normal contact between anoxic and pre-anoxic sedi ments was not recovered. However, two cores (below 3,150 m 05 and 06 GC) recovered oxic pelagic sediments of middle an late Pliocene age unconformably beneath Holocene anoxic sedi ments and in a breccia, respectively.

Abundant gypsum in cores and dredge hauls was the mos surprising discovery at Bacino Bannock. In cores, individua crystals range in size from microscopic to the full diameter o the core barrel (7 cm). The dredges recovered enormous masse of interlocked gypsum crystals, up to 0.5 m across (Fig. 3). Botl dredges were caught up in the gypsum and required more tha 5 tons of pull and several hours (more than 24 h at station 11 D to be freed. These composite gypsum masses were only recovere from the steep western wall of the central bulge where the adhered to a hard substrate of grey Pleistocene marlstone individual crystals in cores and dredges were both tabular an bipyramidal and many contained inclusions concentrated alon crystal planes. Inclusions consisted of pelagic debris such a planktonic foraminifera (Fig. 4), including *Globigerina bulloide Globigerinoides ruber* and *Orbulina universa*, pteropods, includ ing the Holocene species *Cavolinia gibbosa*, and *Cavolini inflexa, Clio pyramidata* and *Atlanta* sp., and mineral grains.

Rubbery mats, similar to those described from the Tyro Basin and consisting of organic fibres containing diatoms, planktoni foraminifera and mineral grains, were found in both cores an dredges. In cores, the mats were recovered as horizontal disk presumably cut from larger mats as though by a biscuit cutte in dredges they occurred between crystals, with no preferre orientation.

CTD measurements indicate normal Mediterranean deep water down to 3,000 m (the length of the conducting hydro-cabl on board). A 15-kHz echosounder recorded horizontal reflector within the water column at depth of 3,228, 3,428 and 3,325 m suggesting locally-high gradients in temperature, salinity and/o particulate matter. During coring and hydrocast operations, th depth of the instrument was monitored by an acoustic pinge

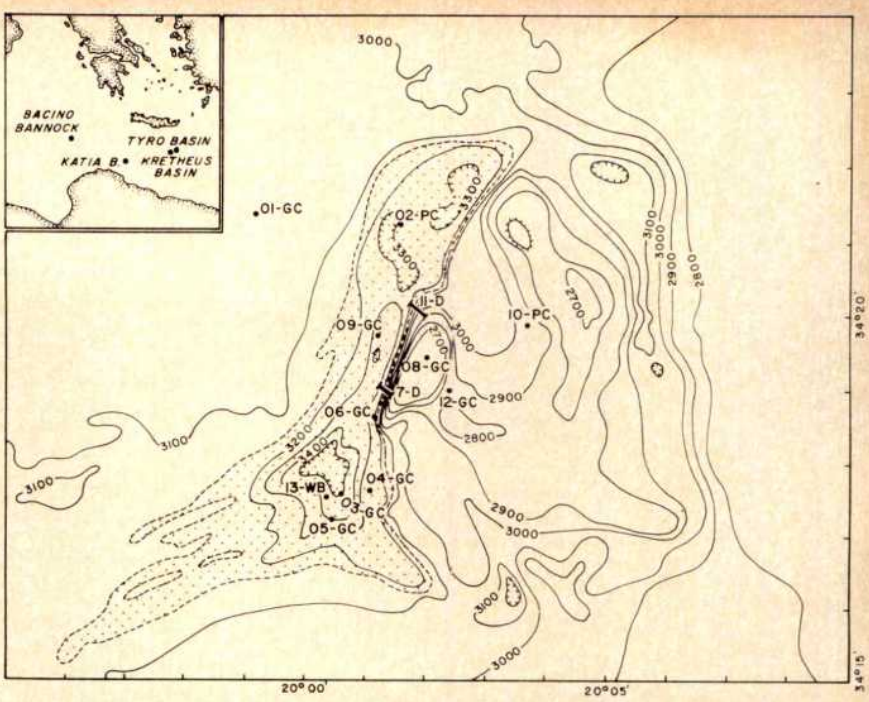


Fig. 1 Bathymetry of Bacino Bannock as determined by 3.5-kHz narrow-beam echo sounder and Seabeam. Contour interval is 100 uncorrected metres. Sampling stations are: D, dredge; GC, gravity core; PC, piston core; WB, water bottle cast. The brine (light stipple pattern) is confined beneath the 3,150-m contour. Navigation is by Loran-C, adjusted to satellite coordinates. Note location of Tyro and Kretheus Basins, where anoxic bottom conditions were discovered last year[3] and of Katia Basin, where such conditions were found during the present investigation.

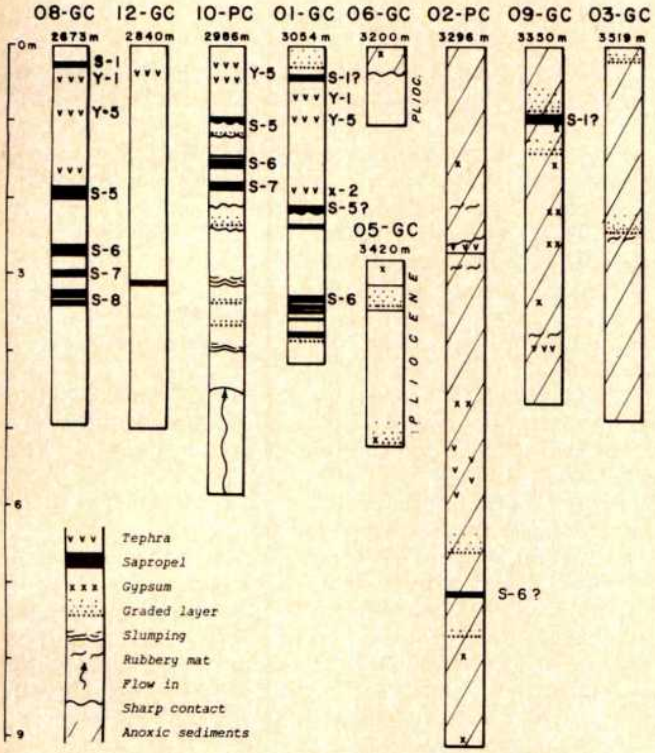


Fig. 2 Simplified core logs from Bacino Bannock. Cores shallower than 3,000 m were recovered from a normal eastern Mediterranean pelagic sequence including sapropels and tephras, whereas cores deeper than 3,200 m were recovered from grey and black sediments.

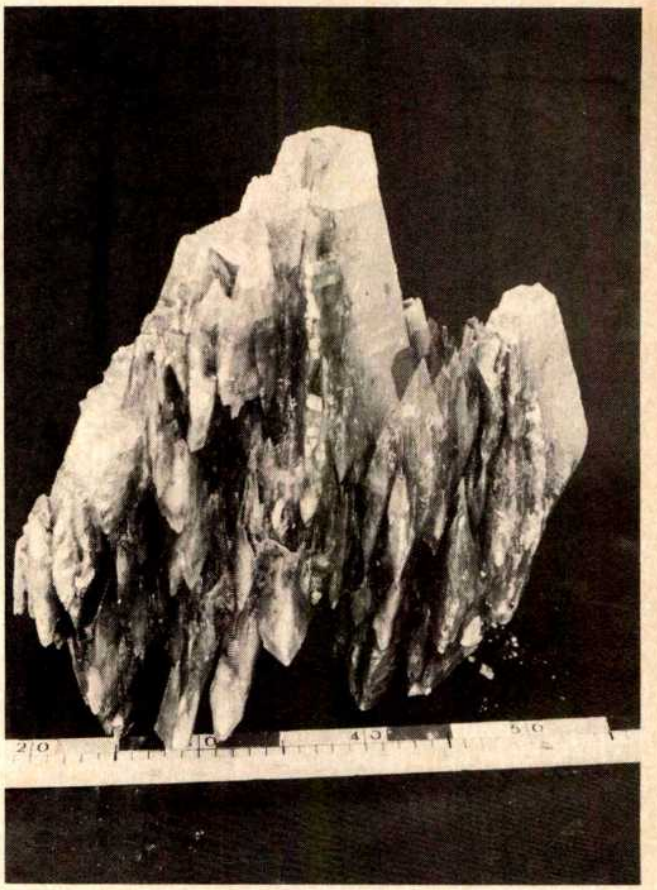


Fig. 3 Photograph showing a large mass of interlocked gypsum euhedra recovered in dredge 7-D (scale in cm). The size and perfection of the euhedra suggest that this gypsum was recently crystallized from a hypersaline brine rather than being relict from the Messinian.

displayed on a facsimile recorder as a depth versus time plot. As the pinger neared the bottom of the basin, a break in the slope appeared on the plot, which we believe resulted from an abrupt change in sound velocity occurring somewhere between 3,100 and 3,340 m.

We infer from sound velocity contrast measurements that the black muds, stinking of hydrogen sulphide, are best explained by the presence of anoxic hypersaline bottom water as has been recovered from the Tyro Basin[3,4]. The upper level of the hypersaline brine is inferred to be at the level of the shallowest closed contour, 3,150 m. This is in agreement with echosounder mid-water reflectors, sound velocity contrast and water depths from which anoxic and oxygenated sediments were recovered. The estimated depth of the interface is consistent with CTD observations that showed no increase in salinity down to 3,000 m. The bottom-water layer occupies an area of 36 km^2 and has a volume of ~4 km^3.

The most probable source of hypersaline bottom water is by leaching from latest Miocene (Messinian, approximately 6–5 Myr BP) evaporites. Although we did not sample strata of this

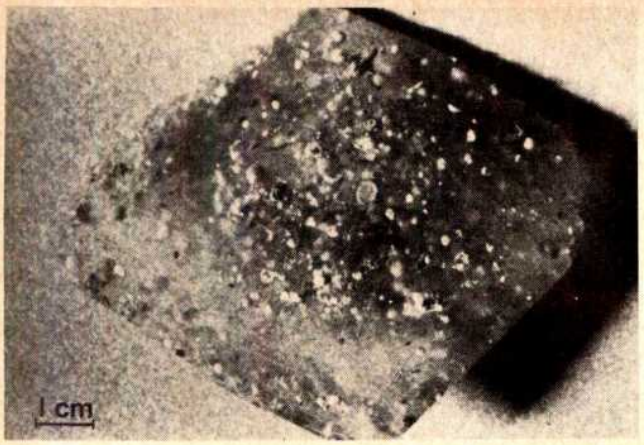


Fig. 4 Photograph showing a euhedral gypsum crystal recovered in core 09 GC, Sec. 1, 80 cm from core top. The numerous biogenic inclusions consist of planktonic foraminifera.

age, several pieces of evidence suggest that Messinian sediments must be at, or close to, the sea floor. First, a single channel reflection profile 10 km west of Bacino Bannock shows a regional reflector (layer M) that correlates with the top of Messinian evaporites[5,6] at sub-bottom depth of 100 m. Second, sedimentation rates calculated for the plateau cores (01, 08 and 10) range from 1.5 to 2 cm kyr^{-1}. Extrapolating this sedimentation rate to 5 Myr BP gives a maximum thickness of 100 m for the Plio-Quaternary section.

At present, gypsum appears to be precipitating from the hypersaline brine at the seafloor. This is indicated by the size and perfection of the crystals, as well as the age of their substrates and inclusions. Gyspum found down-core could have precipitated either from bottom water, before burial at the sediment-water interface, or from pore water, after burial within the sediment.

The degree of anoxia and/or hypersalinity may have varied with time as shown by the change in colour intensity down-core, and by the irregular distribution of gypsum crystals. Some of the sediments are indeed re-sediments, as suggested by the occurrence of millimetric silty laminae, high sedimentation rate and the presence of benthic foraminifera, mostly displaced down-slope. It is possible that the variations in the degree of anoxia were responses to eastern mediterranean-wide hydrographic conditions, as well as to local phenomena. A jet-black layer at 711 cm in core 02 PC, which may correlate with sapropel S-6, contains a cold-water fauna dominated by *G. bulloides* and *Globoquadrina dutertrei*. This fauna characterizes the middle Pleistocene 'glacial anoxia' event observed throughout the eastern Mediterranean[7]. The base of this core is referable to the middle Pleistocene *G. oceanica* nannofossil zone.

The Mediterranean Ridge is marked by numerous subcircular depressions, some of which have been interpreted as resulting from dissolution and collapse of Messinian evaporites[8,9]. Thus, it is reasonable to expect strong density stratification and consequently, anoxic bottom waters in other basins. Indeed, Jongsma *et al.*[13] have described anoxic conditions present now in the Tyro Basin, and up to a few thousands years ago in the adjacent Kretheus Basin, both in the Strabo Trench. On *Bannock* cruise 84-12, we also identified anoxic conditions (cores 29 PC and 30 GC) that lasted until a few thousand years ago in basins of the Katia area near the Lybian continental margin.

In conclusion, we note that the unique aspect of Bacino Bannock is the deep water precipitation of the gypsum under very specific geological, chemical, hydrographical and physiographical conditions.

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1. Degens, E. T. & Ross, D. A. (eds) *Hot Brines and Recent Heavy Metal Deposits in Red Sea*, 1-600 (Springer, Berlin, 1969).
2. Erickson, A. J. & von Herzen, R. P. *Init. Rept. DSDP* **42**(1), 857-872 (1978).
3. Jongsma, D. *et al. Nature* **305**, 795-797 (1983).
4. De Lange, G. J. & ten Haven, H. L. *Nature* **305**, 797-798 (1983).
5. Ryan, W. B. F. & Hsu, K. J. *et al. Init. Rept. DSDP* **13**, 175-218 (1973).
6. Hsu, K. J., Cita, M. B. & Ryan, W. B. F. *Init. Rept. DSDP* **13**, 1203-1231 (1973).
7. Thunell, R. C., Williams, D. F. & Cita, M. B. *J. Foraminifera Res.* **13**, 283-290 (1983).
8. Belderson, R. H., Kenyon, N. H. & Stride, A. H. *Geology* **6**, 716-720 (1978).
9. Kastens, K. A. & Spiess, F. N. *Mar. Geol.* **56**, 181-194 (1984).

Abundance variation of iridium and trace elements at the Permian/Triassic boundary at Shangsi in China

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Despite recent searches for anomalous iridium(Ir) concentrations at the Permian/Triassic boundary (P/Tr) of marine sediments[1-3], no Ir above a level of 0.5 p.p.b. (parts per 10^9) has been found. Detailed event–stratigraphical research has revealed a comparatively high platinum-group enrichment and geochemical anomalies of trace elements in the P/Tr boundary clay at Meishan, Changxing County, Zhejiang Province, China[4]. We report here an investigation of Ir and trace-element abundances in the P/Tr boundary layer of Shangsi section, Guangyuan County, Sichuan Province (Fig. 1). An Ir value of 2 p.p.b. detected in the boundary layer supports the occurrence of an extraterrestrial event at the end of the Permian.

The Shangsi P/Tr layer is located in a road section ~1 km from the town of Shangsi. This section has been extensively investigated, so that stratigraphical sequences of the Permian and Triassic are clear. However, opinions differ as to the demarcation of the P/Tr boundary; most hold that the boundary is close to the horizon where sample AG253 was collected[5].

We concentrated on the P/Tr boundary sediments of total thickness 4 m and most of the samples were collected from shales and clay. The stratigraphical sequence of measured section is shown in Fig. 2.

Note that in South China, *Pleuronodoceras* sp., *Pseudotirolites asiaticus* and *Neogondolella changxingensis* in layer 3-5 are index fossils usually present at the top of the Changxingian. However, a new species of *Claraia* was also found. Hence, the different fauna groups provide several options on where to put the P/Tr boundary in the Shangsi section. For instance, the base of the Triassic has been placed between layer 2 and 3, according

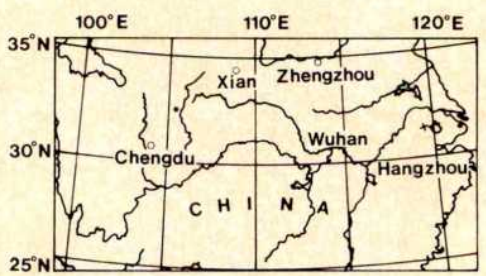


Fig. 1 Sketch map of the locations of two Permian/Triassic sections. *, Shangsi section, Guangyuan; ×, Meishan section, Changxing.

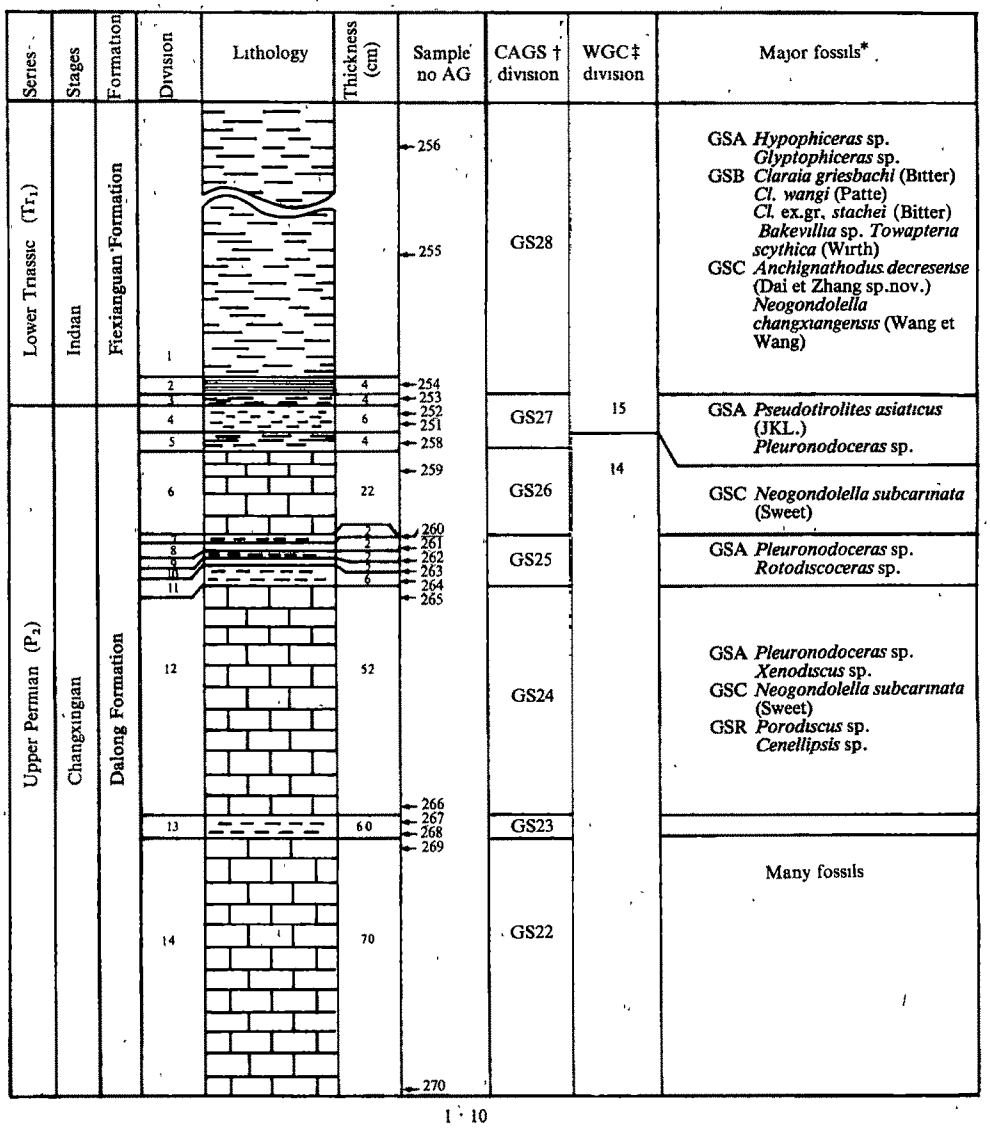


Fig. 2 P/Tr boundary stratigraphical section at Shangsi, Guangyuan. *, From Li Zishun; †, Chinese Academy of Geological Science; ‡, Wuhan College of Geology. Divisions: 1, silty shale; 2, grey shale; 3, grey-black calcareous shale; 4, light coloured shale; 5, grey-black calcareous shale; 6, grey siliceous limestone; 7, grey shale; 8, dark-grey calcareous shale; 9, grey shale; 10, dark-grey siliceous shale; 11, grey shale; 12, dark grey siliceous limestone; 13, grey shale; 14, dark-grey siliceous limestone.

to the ammonites. But, from the bivalves, the P/Tr boundary is lower, whereas conodont assemblage favours moving the P/Tr boundary much higher.

We analysed 10 samples from Shangsi P/Tr section by instrument neutron activation analysis (INAA); the analytical results are shown in Table 1. To check the reliability of INAA, some samples of Cretaceous/Tertiary boundary clay from several sections in Western Europe were also measured. The results seem to concur.

From the 10 analysed samples, only one (AG253) shows an Ir concentration of 2.0 ± 0.5 p.p.b., which is based on two laboratory runs, the lower limit of detectable Ir in the analyses is about 0.5–0.6 p.p.b. Note, that the Shangsi samples, investigated by Zhang *et al.*[3], were taken from layers GS25 and GS24 (equivalent to layers 11 and 12 in the present study), which lies ~0.5 m lower than sample AG253.

Table 1 shows the data sets of the abundance variation of 14 other elements. There are two variation trends: elements of the

Table 1 INAA results of samples at Shangsi section

Element	AG256	AG254	AG253	AG252	AG251	AG258	AG260	AG262	AG264	AG268
Ir	ND	ND	2.48	ND	ND	ND	ND	ND	ND	ND
Ni	42.1		63.2			43.8		46.9	38.3	35.9
Co	15.2	25.8	17.5	2.43	1.83	21.6	5.99	7.25	4.13	1.52
Fe	3.25	2.43	3.62	1.63	1.57	2.29	2.40	2.21	1.96	1.75
Cr	64.1	71.9	88.7	7.85	3.80	57.1	4.98	6.79	2.18	3.50
Sb	0.81	0.91	1.2	0.57	0.47	1.18	0.36	0.38	0.45	0.18
U	2.31	4.08	5.56	9.46	9.11	4.49	4.68	6.95	11.9	13.3
Th	11.1	12.4	13.6	39.5	42.2	13.8	38.8	47.2	49.5	54.5
Rb	136	73.9	162	126	114	107	119	116	119	115
Cs	7.70	4.66	9.64	13.4	11.0	6.09	17.9	12.0	11.4	12.0
Sr	104	1010	160	540	574	875		158	168	113
Ba	294	180	314	70	55.5	235	159	71.1	111	141
Zr	270	198	349	476	610	270	326	936	1020	693
Hf	3.68	3.49	3.71	9.69	10.1	4.20	7.35	17.3	16.7	12.7
Ta	0.91	0.69	0.96	1.68	1.67	0.87	2.11	2.22	2.58	3.39

ND, Not detected; all measurements are p.p.m. except Ir (p.p.b.) and Fe (%).

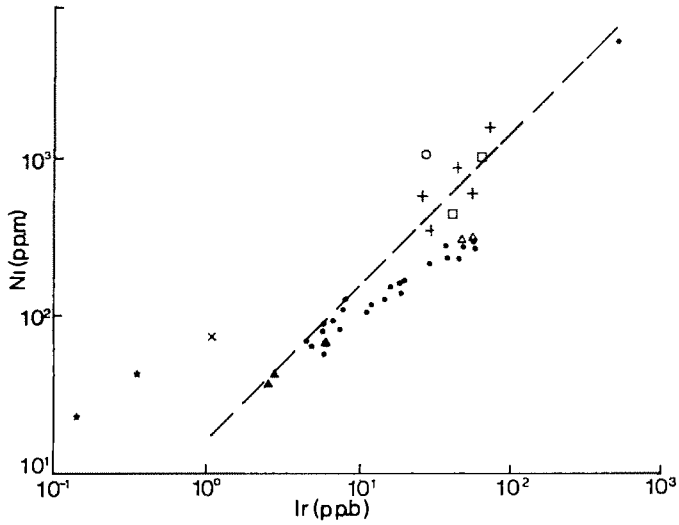


Fig. 3 Relationship between Ni and Ir content in the boundary rocks of the Permian/Triassic (P/Tr); Cretaceous/Tertiary (K/T) X, Crust; *, CI; †, Tunguska metallic spheres[6]; ▲, P/Tr boundary beds at Shangsi; ★, average Danish K2 E1 rocks (from ref. 8); ●, average Danish K/T boundary bed (from ref. 9); ⊙, average Danish K/T boundary bed (from ref. 8); □, average Danish K/T boundary bed (from ref. 7); △, average Danish K/T boundary bed (from ref. 10).

first trend reach maximum or sub-maximum values at AG253. This is the situation for siderophile elements (Ni, Cr, Fe), chalcophile (meteorite) elements (Sb) and a few lithophile elements (Ba, Rb). Most of the lithophile elements (Cs, Rb, Hf, Ta, Zr, Th, U and so on) belong to the second variation trend, which shows decreasing elemental concentrations in sediments of younger geological ages. So, there is probably little or no correlation between the Ir anomaly and the variation trend of most lithophile elements. However, the variation of Fe, Ni, Cr and so on shows a close correlation to the Ir anomaly in sample AG253.

Ganapathy[6] has used the Ni/Ir ratio to distinguish metallic microspherules of an extraterrestrial origin from those of terrestrial origin: on an Ni–Ir diagram, microspherules of extraterrestrial origin show regular linear distribution. Figure 3 shows the Ni and Ir contents of CI carbonaceous chondrites, Tunguska microspherules and Danish Cretaceous/Tertiary boundary clay. These points tend to an approximately linear distribution. The slope of the dashed line in Fig. 3 reflects the cosmic ratio of Ni and Ir. The Ni/Ir points of Shangsi and Meishan samples plotted on Fig. 3 fall into a linear distribution area. Figure 3 also shows that the Ni and Ir values of the crust, Upper Cretaceous beds and Lower Palaeocene beds from Denmark deviate from the linear distribution.

The evidence from the mass extinction of biocommunities, the Ir anomaly of AG253, the variation in the abundance of trace elements, and the comparison between the platinum anomalies at P/Tr boundary at Meishan, Changxing[4] and those at the end of Cretaceous throughout the world, supports the conclusion that the 2 p.p.b. Ir anomaly in AG253 reflects the effects of an extraterrestrial event that occurred at the end of Permian.

Study of the Ir anomaly may provide a new approach to stratigraphical subdivision and correlation. The above arguments place the P/Tr boundary at the Shangsi section at the base of layer 3.

This paper is only a preliminary report and more detailed studies are being planned.

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1. Asaro, P *et al. Geol. Soc. Am Spec. Pap* **190**, 517–528 (1982)
2. Alekseev, A S. *et al* in *Abstr. 14th Lunar planet Sci Conf* 3-4 (NASA, 1983)
3. Zhang, J *et al Acta Petrol Miner Analyt* **2**, 81–86 (1983)
4. Sun, Y Y *et al* in *Development in Geoscience* (ed Tu Guangzhi) 235–245 (Academia Sinica, Beijing, 1984)
5. Li, Z. *et al An excursion guide to the Permian–Triassic Boundary section, Guangyuan, Sichuan Province* (in the press)
6. Ganapathy, R *Science* **220**, 1158–1161 (1983)
7. Alvarez, W *et al Geol Soc. Am Spec. Pap* **190**, 305–316 (1982)
8. Smit J *et al Nature* **285**, 198–200 (1980)
9. Kyte F T *et al. Nature* **288**, 651–656 (1980)
10. Ganapathy, R *Science* **209**, 921–923 (1980)

Timing of deglaciation from an oxygen isotope curve for Atlantic deep-sea sediments

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The exact timing of deglaciation is an important issue in the context of climatic feedback involving the deglacial rise in atmospheric CO_2 seen in ice cores[1–4]. We have produced evidence, from box cores taken in the equatorial Atlantic, that the last deglaciation occurred in two major steps which were separated by a brief pause[5]. Here we propose that this pause is equivalent to the period of glacial readvance known as 'Younger Dryas', which lasted from about 11 to 10 kyr BP. If our correlation is correct, then the initiation of deglaciation occurred after 14 kyr BP, which disagrees with most other deep-sea timescales.

Two-step deglaciation has been proposed previously, notably by Duplessy *et al.*[6] who based their conclusions both on extensive micropalaeontological work at the Institut de Géologie du Bassin d'Aquitaine, and on new radiocarbon-dated $\delta^{18}O$ signals measured at Gif-sur-Yvette. Berger[7] proposed that the Atlantic deep-sea deglaciation record is correlated, as a two-pulse feature, with the record in the Gulf of Mexico[8] which clearly shows a double-pulse meltwater input[9]. Independently, Ruddiman and McIntyre[10] came to the conclusion that deglaciation occurred in major pulses, based on the micropalaeontological record in North Atlantic sediments.

The pulsed nature of the last deglaciation, then, appears to be well established. However, strong disagreement persists concerning the matter of timing[11,12]. Several papers[8–10] date the beginning of deglaciation well before 15 kyr BP. In contrast, our data from the Pacific suggested that deglaciation commenced after this time[13]. Also, the data given for the Gulf of Mexico by Emiliani *et al.*[14] show no major influx there before 14 kyr BP. The timescale of Duplessy *et al.*[6] has deglaciation beginning near 15,000 BP. Sarnthein *et al.*[15] suggest that "the Termination I continued from about 16,000 to 8,500 yr BP", but consider that tropical planktonic foraminifera "indicate a delayed ocean warming which did not start till 14 kyr BP", in the Northern Hemisphere.

The chief problem with accepting any of the published timescales is the varying quality of radiocarbon ages in deep-sea sediments; despite special precautions, problems persist. The well-dated core of Duplessy *et al.*[6] (Core CH 73139C, west of Ireland), for example, shows virtually identical radiocarbon ages for the section between 110 cm and 145 cm, namely, 11.5–11.7 kyr, as well as a major break in sedimentation rate near 200 cm. These results suggest that the record suffers from redeposition events and/or other disturbances. Although the ages down to Ash Layer 1 appear correct (the date of this layer is near 10 kyr), the older ages downcore are suspect. Within the crucial interval centred on 15 kyr, little or no planktonic carbonate may have been delivered to this site because of its northern position[10].

Our box-core data (Fig. 1) allow an independent assessment of the reliability of bulk carbonate ages. For this purpose, the cores are correlated on the basis of oxygen isotopes, with support

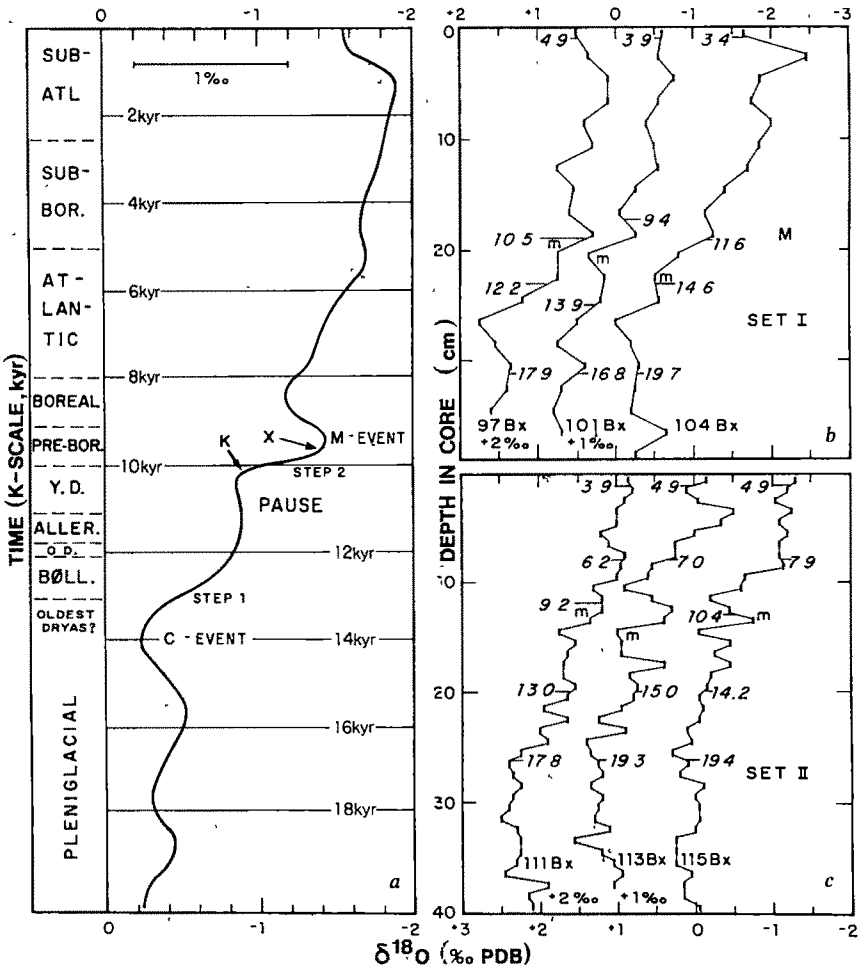


Fig. 1 Timescale of deglaciation based on stacked $\delta^{18}O$ record of three equatorial cores from the Atlantic (*a*). K, the fixed point, taken as end of Younger Dryas, at 10.2 kyr X, The level where radiocarbon ages of bulk carbonate are 11 kyr on average. The K-timescale is based on position of point K and linear sedimentation rate. *b*, $\delta^{18}O$ records from three box cores from the equatorial Atlantic, and ^{14}C dates in kyr. m, Appearance of *G. menardii*. M-event, a 'warm' excursion in the $\delta^{18}O$ record at the end of deglaciation thought to be correlative with the Gulf of Mexico meltwater event of refs 8, 9, 14. *c*, Similar to *b* but cores from tropical South Atlantic. $\delta^{18}O$ measures against PDB standard (*G. ruber*, pink).

from the appearance of the planktonic foraminifera *Globorotalia menardii*, a Holocene marker species. The data plotted in Fig. 1*b,c*, demonstrate that there are two sets of cores whose sedimentation rates are almost identical. Within each of these sets, correlation coefficients are very high (≥ 0.9). They are diminished when differences in overall sedimentation rates are assumed, as we established by systematic numerical experiments. Depth levels within these sets, then, are more or less synchronous. The synchroneity, presumably, tends to deteriorate with depth-in-core, as small differences in sedimentation rates lead to increased divergence in age. If we accept synchroneity within sets for Holocene sediment (<11 kyr), a typical standard deviation about the mean of radiocarbon ages is 0.5–0.9 kyr (Table 1, second and fourth column). This uncertainty seems to reflect the confidence which can be put in the dating of isotope signals based on bulk radiocarbon in these cores. The scatter is a result of sampling and counting errors, mixing processes, and redeposition of fine-grained carbonate. These radiocarbon age data, quite clearly, cannot be used for arguments involving time-resolution of <1,000 yr.

In the following analysis, we use the radiocarbon dates for general guidance: the detailed timescale we propose is based on the correlation of climatic signals. We argue that the radiocarbon dates must be considered maximum ages. Our chief evidence is the high average age of surface sediments (4.1 and 4.6 kyr, Table 1). High surface ages have been reported previously from deep-sea carbonates, they were explained by benthic mixing processes[16,17]. To a first approximation the age of the mixed layer represents the residence time of particles in the mixed layer reservoir, whose thickness should be close to $s \times t$, where s is the sedimentation rate and t the age. For our cores ($s = 1.5$ to 2 cm kyr^{-1}) this thickness would be between 7 and 8 cm. Such a high value conflicts with expectations for these areas[18], especially for the set II region where ocean productivity is low and benthic stirring therefore reduced[19]. We suggest that the old surface ages result not from vertical benthic mixing so much as from the lateral advection of old fine-grained carbonate, delivered from elevated areas. Direct evidence for 'old' fines is in the comparison of coarse fraction ages with bulk ages (Table 2). The bulk ages are older by about 2 kyr, on average. The fines, then, must be older than the coarse material by 2.5 to 3 kyr. Discrepancies in the ages of coarse and fine fractions of deep-sea carbonates have been reported previously[20,21]. The point is that the climatic signals, carried in the isotope composition of planktonic foraminifera, is embedded within sediment that is too old, as far as the signal's timescale is concerned.

Table 1 Average ^{14}C ages (in kyr) at selected depth levels, in cores of set I and set II, and comparison with K-scale ages (based on climatic record)

Depth (cm)	Set I	K-age (Δ)	Set II
1	4.1 ± 0.8	0.5 (3.6)	4.6 ± 0.5
8			7.0 ± 0.9
19	10.9 ± 0.6	9 7 (1.2)	13.3 ± 1.1
25	14 4 ± 1.4	12.8 (1.6)	18.0 ± 0 9
31	18.1 ± 1.5	15.9 (2.2)	

K = 10.2 kyr; M = 9.5 kyr; Δ = difference between set I age and K age.

Table 2 Radiocarbon ages (in kyr) of bulk carbonate and coarse fraction in INMD 115 Bx

Depth (cm)	Bulk age	Coarse fraction	Fine fraction*
0–2	4.85 ± 0.17	2.24 ± 0.11	5.62
7–9	7.87 ± 0.12	6.90 ± 0.13	8.11
19–21	14.17 ± 0.19	11.4 ± 0.17	14.93

* Fine fraction calculated (based on % carbonate and % sand): carbonate, 90.7, 91.4, 89.5; sand, 20.7, 18.3, 19.4.

We now consider the land-based timescale of climatic change for deglaciation and the Holocene to date our isotope signal (Fig. 1*a*). The resulting timescale should be younger than the corresponding deep-sea radiocarbon ages, by an amount comparable to the difference between bulk- and coarse-fraction ages. Of course, the line-up of climatic changes on the land and in the sea should match closely.

Figure 1*a* is derived from stacking the signals of cores 97, 101 and 104 (Fig. 1*b*). The shape of the isotope signal is very similar to one published earlier, from the same region, based on Albatross cores[22], but without age control. The radiocarbon measurements suggest an age near 11 kyr for step 2 (point X). According to our argument, this age represents a maximum for this climatic event. The most nearly synchronous and also the younger event on land, marking a time of rapid change from cold to warm conditions, is represented by the transition from the Younger Dryas cold period to the Preboreal warm interval. Thus the 'cold spot' preceding step 2 in our signal is fixed as the Younger Dryas, which is centred on 10.5 kyr. The entire timescale is now derived by assuming zero age for the surface, and linear sedimentation rate to the Younger Dryas fix-point. With this assumption, an excellent fit is obtained with the Norden timescale[23], based on Scandinavian climatic history (Fig. 1). The differences between the set I ages and the ages found by climate correlation are of the expected magnitude (except for the benthic mixed layer proper) (Table 1, third column).

Our new timescale puts the start of deglaciation just after 14 kyr BP (instead of 15 kyr, based on raw ^{14}C data). Step 1 is centred on 13 kyr and step 2 on 10–9.5 kyr. Our deglaciation steps compare favourably with the micropalaeontological 'warming' steps of Ruddiman and McIntyre[10], established for the northern Alantic. The correlation of the deglacial pause with the Younger Dryas, a major cold event on land, was previously suggested by Duplessy *et al.*[6]. The end of the pause is at point "K", which is taken to be 10.2 kyr BP. This event fixes our timescale, by linear interpolation and extrapolation. Point K slightly predates the Ash Layer 1 of Ruddiman and McIntyre[24], dated at 9.8 kyr BP in ref. 6.

We refer to the timescale adopted in Fig. 1 as the 'K-scale'. Its veracity ultimately rests on a climatic correlation (hence K for Klima), namely, the fit of the pause between the two deglaciation steps to the Younger Dryas cold period. This correlation was first suggested to us by Johnson[25].

Major questions arising from the new deglaciation scale are: what is the positive feedback mechanism which was responsible for the steepness of the two steps; why did deglaciation pause halfway through its completion; and is the published information for ice retreat on land compatible with the deep-sea oxygen isotope record when dated in the manner shown? The evidence for pulsed meltwater input, combined with the short timescale advocated here, makes it likely that the sought-for feedback mechanisms involved intermittent slowing of vertical ocean mixing (the 'Worthington effect')[26]. Such slowing would affect the carbon dioxide content of the atmosphere[27], and hence the direction and the rate of climatic change. There is one possible way the 'early-deglaciaton' timescale[10,15] could be resurrected: if it could be shown that lack of carbonate production (for example, owing to a meltwater lid) produced a carbonate hiatus in the crucial period, near 15–16 kyr, so that a planktonic isotope signal was not recorded, even in the equatorial Atlantic.

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1 Delmas, R J, Ascencio, J-M & Legrand, M *Nature* **284**, 155–157 (1980)
2 Neftel, A, Oeschger, H, Schwander, J, Stauffer, B & Zumbrunn, R. *Nature* **295**, 220–223 (1982)
3 Broecker, W *Geochim. cosmochim Acta* **46**, 1689–1705 (1982)
4 Berger, W H & Keir, R S in *Climate Processes and Climate Sensitivity* (eds Hansen, J E Takahashi, T) 337–351 (American Geophysical Union, Washington DC, 1984)
5 Berger, W H, Killingley, J S, Metzler, C V & Vincent, E *Quat Res* (in the press)
6 Duplessy, J C, Delibrias, G, Turon, J L, Pujol, C & Duprat, J *Palaeogeogr, Palaeoclimatol, Palaeoecol.* **35**, 121–144 (1981)
7 Berger, W H in *Climate in Earth History* (eds Berger, W H & Crowell, J) 43–54 (National Academy Press, Washington DC, 1982)
8 Kennett, J P & Shackleton, N J *Science* **188**, 147–150 (1975)
9 Leventer, A, Williams, D F & Kennett, J P *Mar Geol* **53**, 23–40 (1983)
10 Ruddiman, W F & McIntyre, A *Palaeogeogr, Palaeoclimatol, Palaeoecol* **35**, 145–214 (1981)
11 Kerr, R A *Science* **221**, 143–144 (1983)
12 Ruddiman, W. F & Duplessy, J-C *Quat Res* (in the press)
13 Berger, W H *Sver geol Unders, Afh* C76, 270–280 (1982)
14 Emiliani, C *et al. Science* **189**, 1083–1088 (1975)
15 Sarnthein, M, Erlenkeuser, H & Zahn, R *Act Colloq Int CNRS, Bordeaux, Bull. Inst Géol Bassin d'Aquitaine* **31**, 393–407 (1982)
16 Peng, T-H, Broecker, W S, Kipphut, G & Shackleton, N. in *The Fate of Fossil Fuel CO_2 in the Oceans* (eds Andersen, N R & Malahoff, A) 355–373 (Plenum, New York, 1977)
17 Berger, W H & Killingley, J S *Mar Geol* **45**, 93–125 (1982)
18 Jones, G A & Ruddiman, W F *Quat Res* **17**, 148–172 (1982)
19 Somayajulu, B L K, Sharma, P & Berger, W. H *Mar Geol.* **54**, 169–180 (1984)
20 Suess, H. E *Science* **123**, 355–357 (1956)
21 Eriksson, K G & Olsson, I U *Bull. Geol Inst Uppsala* **42**, 1–16 (1963)
22 Berger, W H *Deep-Sea Res.* **25**, 473–480 (1978)
23 Mangerud, J, Andersen, S. T, Berglund, B E & Bonner, J J *Boreas* **3**, 109–128 (1974)
24 Ruddiman, W F & McIntyre, A *Quat Res* **3**, 117–130 (1973)
25 Johnson, R. F *Scripps Inst of Oceanogr Ref Ser* **80–18**, 1–218 (1980)
26 Berger, W H & Killingley, J. S *J mar Res* **40**, 27–38 (1982).
27 Keir, R S *Earth planet Sci Lett* **64**, 445–456 (1983)

A prehistoric calendrical site in Argyll?

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The hypothesis that many prehistoric standing stone sites in Britain were set up, in relation to natural horizon marks, as astronomical observing instruments has been controversial for the past two decades. Although based primarily on statistical arguments, whether or not it stands will depend on the outcome of practical tests; it must prove able to predict at some sites the occurrence of archaeological features which can then be found by excavation. We report here on the findings at Brainport Bay in Argyllshire of artificial features pointing towards the midsummer sunrise that have been dated suitably early and of another feature indicating alignment towards the sunset at the equinox, that was first predicted and then discovered. These results seem to provide strong support for Thom's hypothesis that calendrically useful solar markers existed in Scotland at least as early as the Bronze Age.

At Brainport Bay, near Minard on Loch Fyne, one of us (P.F.G.) discovered in 1976 a series of artificial stone structures which were first interpreted as a settlement site, but the absence of clear signs of dwellings or of midden material made an alternative hypothesis desirable. Since then, the possibility of their forming an alignment towards midsummer sunrise has been considered, which seemed to explain several hitherto inexplicable features[1,2]. The presence of flint flakes and artefacts and of shattered quartz fragments on the various parts of the site confirmed Mesolithic or Neolithic activity, but there were also much later iron objects and slag. The supposed alignment consists of three main features running from north-east to south-west, namely the 'main outcrop' (with paving on it and two lower revetted 'terraces' at its south-west end), the 'observation boulders', 9 m further south-west and, 50 m further in the same direction, the 'back platform', a few metres higher up (Fig. 1).

From between the two boulders one has a striking view north-east up Loch Fyne, and a partly artificial rock notch at the south-west end of the main outcrop (1.38 m deep and 1.30 m wide at the top) frames the only two distant peaks to be seen—Beinn Oss and Beinn Dubhcraig, 28 miles away near Tyndrum. A small socket was found immediately in front of this notch and another up at the north-east end of the outcrop; two stone slabs 1.28 m and 1.46 m long, which fit these sockets well, were

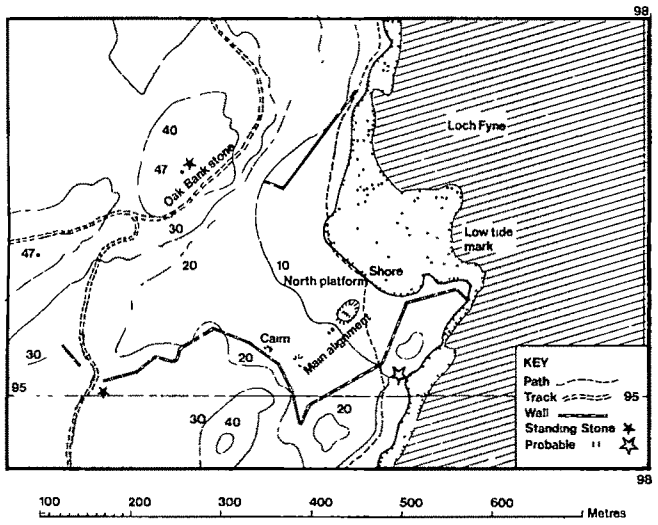


Fig. 1 General plan of Brainport Bay showing the main alignment (with the Back Platform at the south-west) and some nearby outlying features. The contours are in metres above mean sea level.

found on the buried land surface nearby. All these features—boulders, notch and sockets—lie on a straight line pointing to the mountain peaks behind which the midsummer Sun rises (see Fig. 3.5 in ref. 2). The arrangement looks like the sights of a huge rifle aimed at the solstitial Sun, but to be of practical as opposed to ceremonial use, the cleft between the two peaks would have to have been used. Measurements by Thom showed that this cleft has a declination of +23°22′, slightly before the solstice of 1800 BC; then it would have marked the upper limb of the Sun rising on two occasions about 32 days apart, before and after midsummer (see p. 134 and Fig. 3.8 in ref. 2).

The third element in the alignment, the back platform, consists of a rock outcrop on the northeastern edge of higher ground and that has been modified with revetting and paving; there is a broad flat area behind it. From here there is a dramatic, though generalized, view of the midsummer dawn, but not through the rock notch, which is now too low.

Excavations in 1982 revealed a buried sandy soil behind the back platform on top of which were struck flint flakes; a ^{14}C date of 1060±80 BC (GU 1705) was obtained for charcoal associated with the flints, equivalent to the fourteenth century BC in calendar years[3]. The revetted edge of the larger terrace next to the main outcrop was found to lie on the same buried soil (Fig. 2). Thus, the artificial elements of the main alignment have been fairly securely dated to the mid-second millennium BC or earlier. Excavations at the 'observation boulders', however, showed that, contrary to previous belief (see p. 134 and plate 3.6 in ref. 2), these have been in position since the time of the post-glacial high sea level, well before Neolithic times.

As it does not point exactly at the solstice, the calendrical function of the main alignment had to remain speculative, unless another clearly-indicated and reasonably precise alignment could be identified nearby; this could serve as a double check on any solar calendar and would tend to confirm that one was in use. The most probable candidate for a second alignment was the fallen stone on the ridge above Oak Bank, ~240 m north-west of the main alignment.

An attempt in 1981 to discover whether the Oak Bank stone could have been an artificial foresight to mark the midsummer sunset, as seen from the large terrace, resulted in the discovery, as a potential backsight, of the small stone North Platform a few metres north of the main outcrop and in the appropriate position; excavations in 1982, however, showed it to be relatively modern. It then seemed appropriate to test whether the fallen stone on Oak Bank might itself have been the backsight for an alignment.

One of us (P.F.G.) had found previously two unusual rock carvings a few metres south-east of the fallen Oak Bank stone, among the dense firs of a Forestry Commission plantation. Each consists of a pecked cup-mark of standard early Bronze Age type, but with a straight shallow pecked groove running through it (Fig. 3). Carving no. 1 has a groove ~60 cm long, on an azimuth of 130.5/310.5°, and points directly back at the Main Outcrop below. Carving no. 2 has a groove ~40 cm long on an azimuth of ~81/261°, ~2.1° greater than the line defined by the two cup-marks themselves. These directions can be determined to within ~0.5° using a prismatic compass checked against a line of known azimuth.

The many fir trees around the carvings obscure the western and northwestern horizons; the groove of no. 1, however, was found to point at a featureless horizon several degrees to the right of the midsummer sunset position. The near east-west orientation of no. 2 suggested that it might mark an equinoctial line, the backsight for which would be the fallen stone nearby, but the eastern horizon across the loch is flat and featureless. Nothing could be seen of the western horizon at first until the azimuth of the groove was transferred 20 m to the base of the fallen stone; it then pointed almost exactly at a deep V-shaped notch ~1 km away in Siaradh Druim, the 'western ridge'. A metal rod laid in the groove of the rock carving confirmed, after some clearance of branches, that it points at this notch. This notch has an azimuth of 261°32.5′ and a declination of +0°08′ as seen from the stone (perhaps +0°04′, if the trees on the left slope are removed) (Fig. 4).

This position is within the range of the declinations of equinoctial sites identified by Thom, the mean of which is approximately at the 'megalithic equinox' of +0.51° (ref. 4). This is the date that would have resulted if the equinox was defined by counting the days in the year and sub-dividing (the summer half being a few days longer because of the eccentricity of the Earth's orbit (see p. 109 in ref. 4)). The Oak Bank alignment could have defined this megalithic equinox very well, if the Sun's lower limb served as the marker against the horizon. Figure 4 shows the 24′-wide zone of fluctuation—the amount

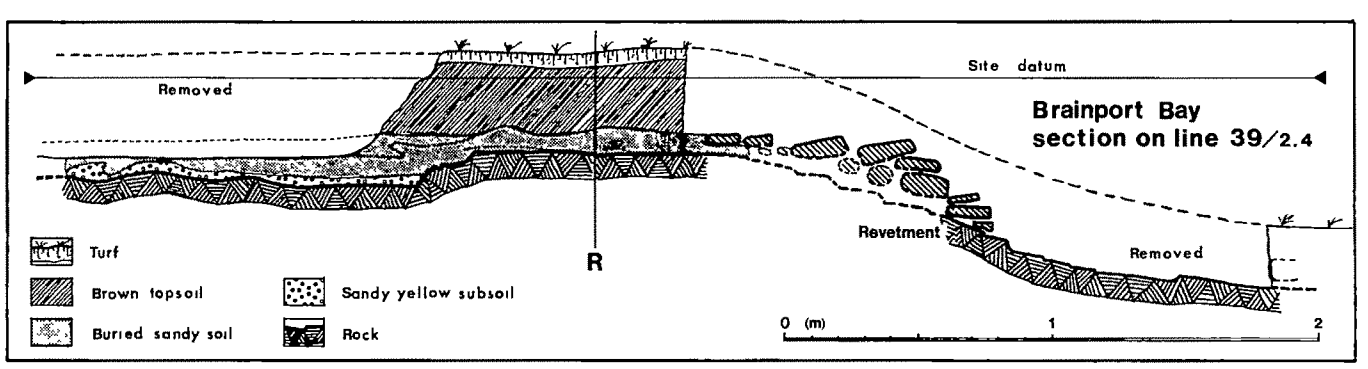


Fig. 2 Section of soil layers on the northwestern edge of the large terrace at Brainport Bay, showing the revetment of the terrace lying on the prehistoric ground surface.

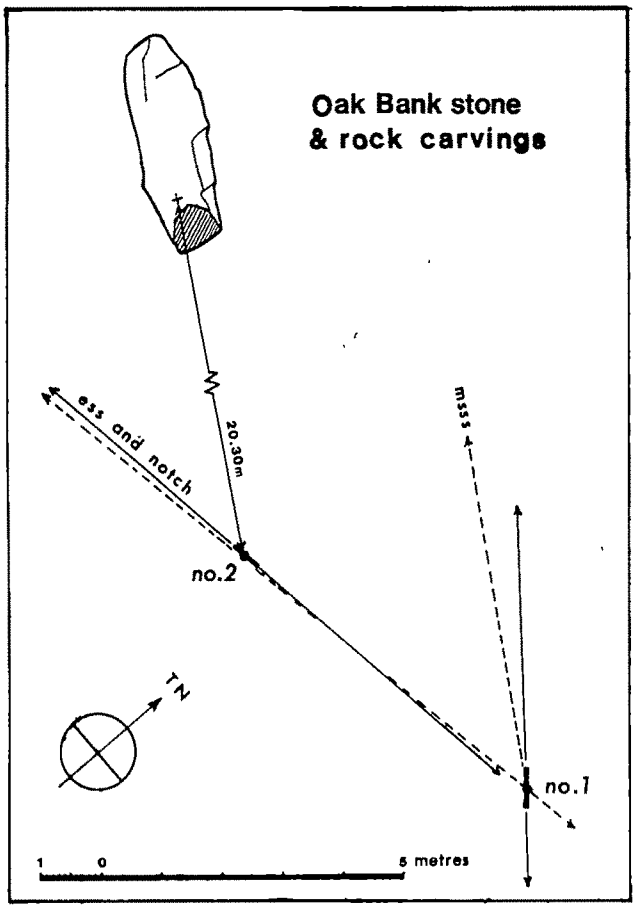


Fig. 3 Plan of artificial features on Oak Bank—the fallen stone and the two rock carvings. Carving no 1 points back to the main site at a distance of 225 m. 'ESS' means equinoctial sunset and 'MSSS' midsummer sunset.

by which the solar declination can vary at sunset at the equinoxes—against the notch. Thus, the first appearance of the full disk in the notch in the spring, and the last in autumn, would have marked the day of the megalithic equinox and the alignment could have served as a good check on a solar calendar.

Ideas about the achievements of prehistoric man in Britain have focused traditionally on his technological and economic skills, deducible directly from the archaeological evidence; ceremonial and religious practices are much harder to recover but seemed to have been suitably primitive[5,6]. However, much evidence from Thom shows that late Neolithic people erected standing stones to mark the position of the Sun and Moon (and some stars) on the horizon at important points of their cycles and that they thereby developed considerable astronomical expertise (see chapter 9 in ref. 4). These ideas have aroused considerable interest and controversy[7-11].

The particular aspect of the Thom hypotheses examined here is that many standing stones marked the 'backsights' (observers' positions) of alignments that could detect when the Sun was at the solstices, equinoxes and at other important intermediate dates of the solar calendar. These lines point towards 'foresights', which are natural marks on the horizon where the Sun rose or set at the times concerned and which are usually indicated in some way by the stones themselves.

The evidence assembled consists of a large number of standing stones, which could have been the backsights for such accurate alignments in Neolithic times, and of the statistical argument that this could not have happened by chance (ref. 12 and see chapter 8 and Fig. 8.1 in ref. 4). There has been much argument about whether such deliberately arranged astronomical lines (on which the more elaborate astronomical hypotheses developed by Thom (concerning the Moon) ultimately depend[4,13,14]) were actually intended to be such by the standing stone erectors,

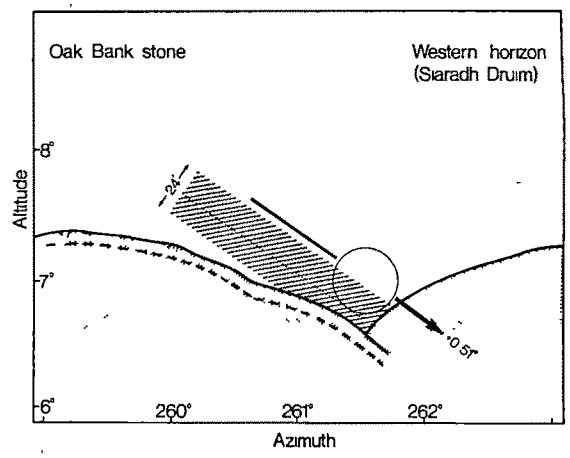


Fig. 4 Siaradh Druim, the 'western horizon', as seen from the Oak Bank stone with the Sun at the 'megalithic equinox'; the dotted line indicates where the real horizon may be, below the tree line. The shaded band shows the range of possible positions for the lower limb at sunset at that time.

about how accurate they were and for what exactly they were used[10].

It can be argued that the genuineness, or otherwise, of the alignments will only be finally settled by testing a selection of examples on the ground, that is, by finding sites where one of the astronomical alignment hypotheses predicts the existence of some feature that can then be sought by excavation[15,16]. One of us (E.W.M.) attempted to do this at the Kintraw standing stone in mid-Argyll, where an artificial platform was discovered, as required by Thom's theory that a precise midwinter sunset alignment existed there[17]. However, as the platform could not be dated, the evidence was inconclusive.

The results from Brainport Bay described here suggest that, at this site, there are two well-marked prehistoric solar alignments, the reality of which has been demonstrated beyond reasonable doubt and which could therefore be interlinked. The conspicuous line towards midsummer sunrise is formed by clearly artificial and by natural features and it has been dated as suitably early, directly, from associated artefacts and indirectly, from a ^{14}C measurement. Yet the discovery that the 'observation boulders' are natural, implies that the alignment began as the chance finding of a suitable site with a long view to the north-east (presumably by people searching for one) and that features on it were modified to form a line towards the midsummer sunrise. This could explain why the 'foresight' mountains, though suitably distant, are not the ideal shape for marking the solstitial sunrise exactly; in other such cases, the Sun's upper limb rose or set along one slope, about parallel to the angle of its diurnal movement (see Fig. 12.2 in ref. 4).

This in turn suggests that the prominent north-east alignment was intended as much for ceremonial as for calendrical purposes; certainly the dawn spectacle is dramatic and could have been viewed by many people. It may also have been a useful calendrical alignment; the date of the solstice could have been found at any period in the past by halving the interval between successive risings in the notch. However, such an indirect alignment is less convincing statistically than one that points exactly at the solstitial declination, hence the search for a second solar line at the site.

Such a solar line, indicating the equinoctial sunset, was found at the Oak Bank stone rock carvings. Because of the obscuring fir trees it is also a good example of the discovery of a convincing alignment through a prediction made from Thom's general solar alignment hypothesis. On such independent testing of potentially accurate astronomical alignments as this depends surely the fate of ambitious re-assessments of the skill and organization of late Neolithic society in Britain[18], and indeed that of the traditional views also[5,6].

We thank the Forestry Commission (Scotland) and, since 1983, Lt Col. R. Gayre of Gayre and Nigg, Minard Castle, for permission to survey and excavate at the site. We also thank the following who assisted at the site: Dr and Mrs J. R. Baker, Mr J. Birdsey, Mr M. Davis, Ms Helen Dyson, Mr H. N. Hawley, Mr H. E. Kelly and Mr S. White. The Director General of the Ordnance Survey is thanked for permission to use the 1:10,000 Ordnance Survey map as the basis for Fig. 1 (Crown copyright reserved).

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1. Gladwin, P. F. *The Kist* **16**, 1–5 (1978).
2. MacKie, E. W. in *Astronomy and Society in Britain during the Period 4000–1500 B.C.* (eds Ruggles, C. L. N. & Whittle, A. W. R.) 111–152 (British Archaeological Rep., British Ser. **88**, Oxford, 1982).
3. Clark, R. M. *Antiquity* **49**, 251–266 (1975).
4. Thom, A. *Megalithic Sites in Britain* (Clarendon, Oxford, 1967).
5. Burl, A. *Rites of the Gods* (Dent, London, 1981).
6. Megaw, J. V. S. & Simpson, D. D. A. *Introduction to British Prehistory* (Leicester University Press, 1979).
7. Atkinson, R. J. C. *Antiquity* **42**, 77–78 (1968).
8. Hawkes, J. *Antiquity* **41**, 174–180 (1967).
9. Thorpe, I. J. in *Astronomy and Society in Britain during the Period 4000–1500 B.C.* (eds Ruggles, C. L. N. & Whittle, A. W. R.) 13–62 (British Archaeological Rep., British Ser. **88**, Oxford, 1982).
10. Heggie, D. *Megalithic Science* (Thames & Hudson, London, 1981).
11. Ruggles, C. L. N. in *Astronomy and Society in Britain during the Period 4000–1500 B.C.* (eds Ruggles, C. L. N. & Whittle, A. W. R.) 153–210 (British Archaeological Rep., British Ser. **88**, Oxford, 1982).
12. Thom, A. *J. R. Stat. Soc.* A**118**, 275–295 (1955).
13. Thom, A. *Megalithic Lunar Observatories* (Clarendon, Oxford, 1971).
14. Thom, A. & Thom, A. S. *Megalithic Sites in Britain and Brittany* (Clarendon, Oxford, 1978).
15. MacKie, E. W. in *Archaeoastronomy in the Old World* (ed. Heggie, D. C.) 117–140 (Cambridge University Press, 1982).
16. Ruggles, C. L. N. *et al. Megalithic astronomy: a new archaeological and statistical study of 300 western Scottish sites* (British Archaeological Rep., British Ser. **123**, Oxford, 1984). 14ff.
17. MacKie, E. W. *Phil. Trans. R. Soc.* A**276**, 169–194 (1974).
18. MacKie, E. W. *Science and Society in Prehistoric Britain* (Elek, London, 1977).

The first pre-Rhaetic therian mammal

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The earliest mammals in the fossil record are recognized by their multicusped and multiple-rooted cheek teeth, and in south-west Britain they appear abruptly in sediments that postdate the Rhaetic transgression at ~200 Myr. Here we report the discovery of teeth of the primitive mammal *Kuehneotherium* in a terrestrial fissure deposit from the Mendip Hills (Somerset, UK) that predates the Rhaetic transgression. The teeth were found in the sediment fill of a collapsed cave[1] at Emborough near Wells during the systematic reinvestigation of a number of late Triassic vertebrate sites. The assemblage is otherwise typically pre-Rhaetic and includes the gliding lizard *Kuehneosaurus*. The finding of mammalian remains throws doubt on the fundamental distinctiveness of pre-Rhaetic and post-Rhaetic vertebrate faunas.

Vertebrate-bearing fissure deposits are known at several sites in the Lower Carboniferous/Mesozoic unconformity either side of the Severn Estuary (Fig. 1). The fissures comprise a variety of features including karstic cave systems, tectonic joints and faults; their fills range from Triassic continental red marls to middle Jurassic marine limestones. In the first major review of the nature and occurrence of fissure deposits, Robinson[1] distinguished between Norian and Rhaeto-Liassic deposits which she believed could be recognized on the basis of their vertebrate faunas[2]. The Norian fauna consisted entirely of sauropsids whereas the Rhaeto-Liassic faunas contained theropsids and mammals in addition to sauropsids. There was also some variation in the nature of the sauropsid fauna, particularly with respect to archosaurs, which occurred only rarely in the Rhaeto-Liassic deposits. This broad division has been accepted by subsequent authors. Typical Norian assemblages include *Clevosaurus*, *Kuehneosaurus*, *Planocephalosaurus*, primitive crocodiles and pseudosuchians[3–5], whereas the Rhaeto-Liassic forms include *Morganucodon*, *Kuehneotherium* and the lepidosaur *Gephyrosaurus*[6–8].

More recently, Marshall and Whiteside[9] have identified marine Rhaetian palynomorphs in sediments at Tytherington Quarry which contain a typical Norian sauropsid fauna, including *Clevosaurus* and *Planocephalosaurus*[10]; this work did not question the existence of two distinct faunas, but threw doubt on the validity of assuming an age prior to the Rhaetic transgression for the sauropsid assemblages. We have confirmed the presence of datable palynomorphs in the Tytherington sequence, but their usefulness is open to question as the deposits are contained within tectonically generated fissures which have suffered more than one phase of disturbance and mixing of the contents.

The Emborough deposit has yielded no palynomorphs and cannot be dated in this way, a problem in common with late Triassic continental sediments elsewhere[11]. The deposit consists of a single large pocket of poorly sorted, locally derived limestone boulders and coarse conglomerate set in a matrix of red marl. The pocket has been interpreted as a collapsed cave deposit[1], and the conglomerate and marl components are typical pre-Rhaetic lithotypes; no Rhaetic or post-Rhaetic clastic components can be recognized. Further evidence of the pre-Rhaetic age of the Emborough deposit is provided by nearby outcrops of basal Rhaetic sediments which overlie a formerly planar transgression surface. This surface now rises southwards, and clearly extended across the top of the Emborough pocket.

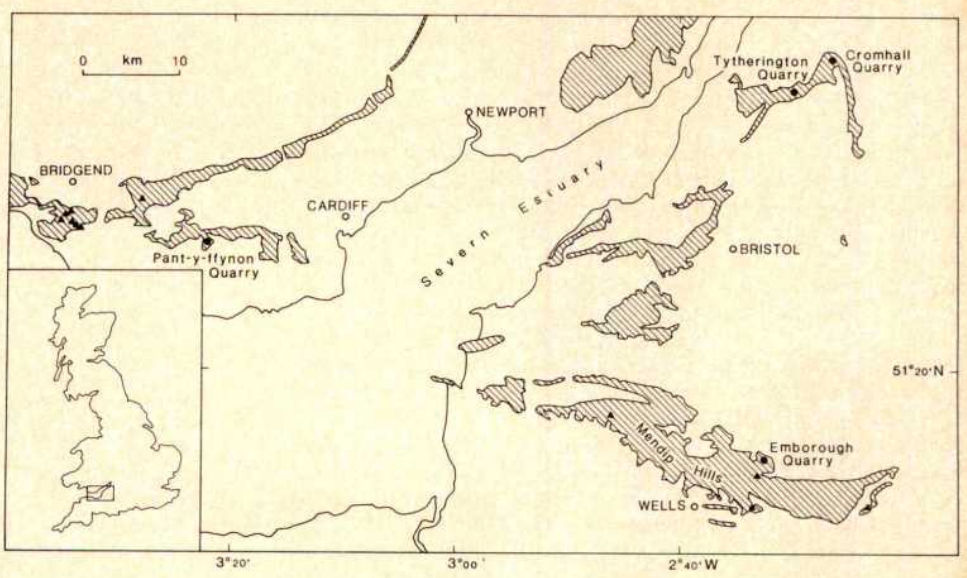


Fig. 1 Vertebrate-bearing Triassic fissure deposits either side of the Severn Estuary, UK. ▨, Carboniferous limestone outcrops; ●, localities mentioned in the text; ▲, other known localities.

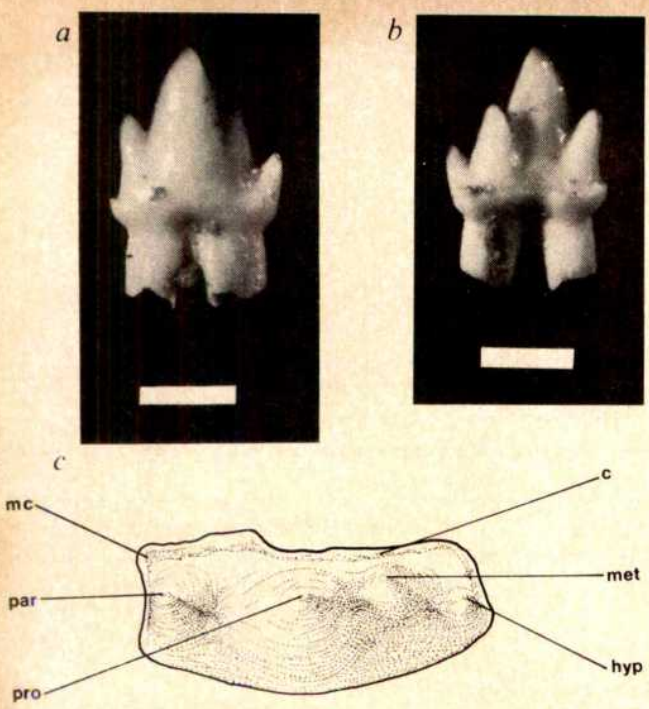


Fig. 2 Lower left molariform tooth of *Kuehneotherium* (AUP no. 11133). *a*, Buccal; *b*, lingual; and *c*, occlusal view. Scale bar, 0.5 mm. c, Cingulum; hyp, hypoconulid; mc, mesial cuspule; met, metaconid; par, paraconid; pro, protoconid.

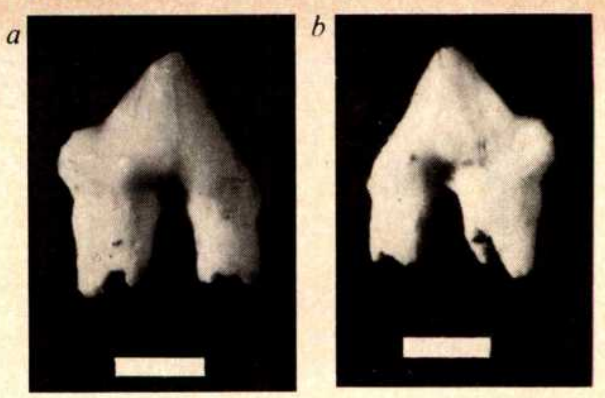


Fig. 3 A premolariform tooth of *Kuehneotherium* (AUP no. 11134). *a*, Buccal; and *b*, lingual view. Scale bar, 0.5 mm.

Following Robinson[1], therefore, we are certain that the Emborough pocket is Norian in age.

The reptilian fauna of Emborough consists of lepidosaurs and archosaurs, and by far the most abundant of these is the gliding lepidosaurian, *Kuehneosaurus*[12]; this genus has also been identified at Cromhall[4] and Pant-y-ffynon[13], although in much smaller numbers. Both of these localities are regarded as pre-transgressional in age[4,14] and the genus is not known at the Rhaeto-Liassic localities.

We have confirmed an abundance of *Kuehneosaurus* at Emborough and have noted sphenodontids. Of the two mammalian teeth, the best preserved (Fig. 2, AUP no. 11133) closely resembles a lower molar of *Kuehneotherium*, but with relatively low central cusps. Molariform teeth of *Kuehneotherium* were first described in detail by Kermack *et al.*[7], who noted that in the lower molars the central cusps were generally elevated and higher than in the upper teeth. However, in one lower left molar described by Kermack *et al.*[7] (BMNH C.855), the cusps are rather less elevated. The best-preserved Emborough tooth is practically identical in this and other respects, indicating beyond doubt that it belongs to the lower left mandible. The second Emborough tooth (Fig. 3, AUP no. 11134) is a premolar showing more polish than the first and it exhibits a well-defined wear facet. Kermack *et al.*[7] emphasized that the premolars of the early mammals showed insufficient characters for a single tooth to be referable to a particular genus, citing the similarity of *Kuehneotherium* premolars to those of *Eozostrodon*. However, the occurrence of the distinct *Kuehneotherium* molar in the deposits indicates that in this instance the Emborough premolar is most probably also from *Kuehneotherium*.

This new record of *Kuehneotherium* from Emborough is important for two reasons. First, it is undoubtedly the earliest record of a therian and may even be the oldest fossil mammal. It predates the kuehneotheriid, *Woutersia*, and the morganucodontid, *Brachyzostrodon*, described by Sigogneau-Russel[15] from the French Rhaetic locality of Saint-Nicholas-de-Port. Although haramyid teeth have been described from earlier deposits, namely the Uppermost Middle Keuper of Halberstadt[16], very little is known of this taxon. While they may be non-therian mammals, considerable doubt remains[17-19] and their systematic position cannot be readily resolved within the framework of early mammals. From an analysis of the tooth wear facets of haramyids, it has been suggested that they may have had an antero-posterior movement of the lower jaw akin to that of the cynodont reptiles[18,19]. Although this group of reptiles is widely accepted as being ancestral to the mammals, both prototherian and therian mammals have a lateral-medial motion of the lower jaw.

Second, the finding of a mammal in deposits with 'typically' Norian assemblages raises serious doubts about the validity of distinctions drawn between pre-transgression and post-transgression terrestrial vertebrate faunas. Although differences in the faunas can be recognized, these may now be no more significant than those between localities of the same age, or even between different sites at the same locality[4].

It seems likely that ecological factors played an important part in determining these assemblages, in particular, feeding habits may have been a factor responsible for mammalian expansion at this time. From the nature of its dentition, *Kuehneotherium* was undoubtedly an insectivore[6,20] but so too were several of its contemporary lepidosaurs[4,8]. This raises the possibility of direct competition, but the reasons for the proliferation of the mammals in Rhaeto-Liassic times need not be related to this, nor were environmental factors connected with the Rhaetic transgression necessarily involved. By means of their implicit endothermy[21], the early mammals such as *Kuehneotherium* may have avoided direct competition with other insectivores by adopting a nocturnal niche.

AUP and BMNH are abbreviations for Aberdeen University Palaeontology and the British Museum (Natural History) collections, respectively. We thank Dr D. J. Batten for palynological advice and Professor K. A. Kermack for his comments on the mammalian teeth. We also thank Mr J. Blatchford for allowing us access to Emborough Quarry. The work presented here forms part of a wider reinvestigation of late Triassic fissure faunas supported by NERC grant GR3/4419, which we gratefully acknowledge.

Received 3 September; accepted 19 December 1984.

1. Robinson, P. L. *J. Linn. Soc.* **43**, 260-282 (1957).
2. Robinson, P. L. *Palaeontology* **14**, 131-153 (1971).
3. Fraser, N. C. *Palaeontology* **25**, 709-725 (1982).
4. Fraser, N. C. & Walkden, G. M. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **42**, 341-365 (1983).
5. Halstead, L. B. & Nicoll, P. G. *Stud. Speleol.* **2**, 93-102 (1971).
6. Kermack, K. A., Mussett, F. & Rigney, H. W. *Zool. J. Linn. Soc.* **53**, 87-175 (1973).
7. Kermack, D. M., Kermack, K. A. & Mussett, F. *J. Linn. Soc.* **47**, 407-423 (1968).
8. Evans, S. E. *J. Linn. Soc.* **70**, 203-264 (1980).
9. Marshall, J. E. A. & Whiteside, D. I. *Nature* **287**, 627-628 (1980).
10. Whiteside, D. I. & Robinson, D. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **41**, 81-85 (1983).
11. Warrington, G. *Proc. Ussher Soc.* **4**, 76-81 (1977).
12. Robinson, P. L. *Proc. geol. Soc.* **1601**, 137-146 (1962).
13. Crush, P. J. thesis, Univ. College London (1980).
14. Crush, P. J. *Palaeontology* **27**, 131-157 (1984).
15. Sigogneau-Russel, D. *Acta palaeont. pol.* **28**, 233-249 (1983); *Zool. J. Linn. Soc.* **78**, 175-186 (1983).
16. Hahn, G. *Palaeontographica* A**142**, 1-15 (1973).

17. Mills, J R. E in *Early Mammals* (eds Kermack, D M & Kermack, K A) 29-63 (Academic, London, 1971)
18 Clemens, W A & Kielan-Jaworowska, Z in *Mesozoic Mammals* (eds Lillegraven, J A, Kielan-Jaworowska, Z & Clemens, W A) 99-149 (University of California Press, 1979).
19 Kemp, T S *Mammal-like Reptiles and the Origin of Mammals*, 363 (Academic, London, 1982)
20 Mills, J R E *J Linn Soc* 50, Suppl 1, 29-63 (1971)
21 Crompton, A W, Taylor, C R. & Jagger, J A *Nature* 272, 333-336 (1978)

Reduction of oak fecundity by low-density herbivore populations

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Explanations for the low levels of defoliation in natural woodlands[1–6] have assumed that insect herbivores have a negligible effect on plant fitness and demography[7,8], perhaps because of plant compensation[9–11]. Levels of defoliation between 5 and 15% are typical, and are attributed variously to the impact of natural enemies, inclement weather and low food quality in keeping herbivore populations at low densities[12,13] (but see refs 14, 15). Here I report the results of a 4-yr study in which matched pairs of oak trees, *Quercus robur*, were sprayed regularly to exclude all herbivorous insects; despite the fact that the unsprayed trees lost only 8–12% of their leaf area, the sprayed trees consistently produced more seeds (from 4.5 to 2.5 times the numbers on unsprayed plants). It is not known whether this substantial reduction in seed production actually leads to a reduction in plant recruitment. It does seem, however, that plant compensation is not fully effective against low-density herbivore populations and, therefore, that slight differences in the level of defoliation between plants may cause important differences in plant fitness.

All invertebrate herbivores were excluded from six pairs of oak trees (matched initially for size, age and date of bud-burst, ranging in girth from 14 to 97 cm). I applied a mixture of contact (permethrin) and systemic (dimethoate) insecticides in a water base, and sprayed the control trees with an identical volume of water. The sprayed trees were further protected by a sticky band around the trunk which excluded walking insects such as the wingless females of the winter moth, *Operophtera brumata*. The sprays were applied monthly from March to September (but every 2 weeks in May and June), at a rate of between 1.5 and 5.5 litres per tree (~500 g active ingredient per hectare). There is no evidence of phytotoxic effects of the spray on the plants, nor is there any suggestion that these particular insecticides act as promoters of flowering or seeding in trees[16]. Regular counts using a variety of methods were made of insect herbivore numbers on nearby trees[17].

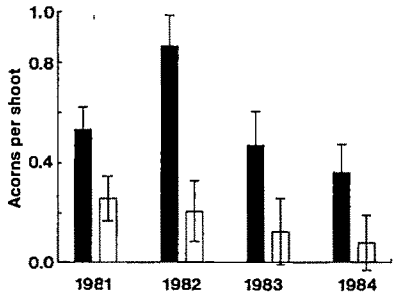


Fig. 1 Number of acorns per shoot on sprayed (solid bars) and unsprayed (open bars) trees in each of 4 yr. The data represent arithmetic means and 5% least significant differences, based on separate 2-way analyses of variance for each year (main effect means calculated over three size classes with two replicates in each class, using the same 12 trees each year).

The numbers of male and female flowers per shoot were assessed from a non-destructive sample of 100 randomly selected shoots per tree in June; defoliation levels, primary and lammas shoot lengths[7] and acorn crop were assessed from a separate sample of 100 shoots in August each year (Table 1).

Insecticide treatment was effective in all years in reducing defoliation levels below 5% and in virtually eliminating gall-forming and sap-sucking insects. Reduced herbivore numbers had no effect on primary or lammas shoot growth, trunk-wood growth or flower production in the following year (Table 1), but led to a significant increase in acorn production (Fig. 1). A variable number of acorns on unsprayed trees were attacked by the gall-forming wasp, *Andricus quercuscalicis* (ref. 18 and R. S. Hails and M. J. C., in preparation). As seen in Table 1, the increase in acorn crop under spraying is not simply an artefact of excluding the gall-former.

In a separate study of 36 trees with girths ranging from 30 to 600 cm, there is no correlation between defoliation and acorn production either within trees (that is, from shoot to shoot) or between trees, in any year of this study. Failure to detect significant depression in fecundity results from the very high tree-to-tree variance in acorn production, and from the fact that certain trees are consistently the most heavily defoliated (the correlation between defoliation in year t and in year $t+1$ is significant for all pairs of years; $n=36$, $r>0.34$, $P<0.05$). Here, the failure to detect subtle ecological effects demonstrates the importance of manipulative field experiments in studies designed to elucidate the role of herbivores in plant population dynamics and as agents of natural selection.

Table 1 Insecticide spraying and reproductive performance in *Quercus robur*

Year	1981			1982			1983			1984		
Treatment	S	U	*P*	S	U	*P*	S	U	*P*	S	U	*P*
Defoliation, %	5.0	10.0	<0.01	3.0	8.0	<0.01	4.0	9.0	<0.01	3.5	11.5	<0.01
Wood increment, see below	0.56	0.54	NS	0.48	0.45	NS	0.39	0.32	NS	0.34	0.34	NS
Primary shoot, cm	11 1	8.4	<0.05	14.0	13.3	NS	11.3	11.8	NS	7.0	6.5	NS
Lammas shoot, cm	9.3	7.8	NS	14.8	14.6	NS	15.6	23.3	<0.05	2.7	4.4	NS
Male flowers, per shoot	0.41	0.44	NS	0.31	0.22	NS	0.39	0.36	NS	0.53	0.64	NS
Female flowers, per shoot	0.61	0.52	NS	0.48	0.33	<0.05	0.79	0 57	NS	0.72	0.69	NS
Peduncles, per shoot	0.30	0.19	NS	0.68	0.34	<0,05	<0.33	0.36	NS	0.32	0.13	NS
Acorns, per shoot	0.53	0 26	<0.05	0.86	0.21	<0.01	0.47	0.13	<0.05	0.36	0.08	<0.01
Acorns + galls, per shoot	0.53	0.26	<0.05	0.94	0.32	<0 01	0.47	0.40	NS	0.38	0.11	<0.05

Arithmetic means for six sprayed (S) and six unsprayed (U) trees divided into three size classes with two replicates per size class. Numbers estimated per shoot are from 100 shoots per tree each year. Wood increment is calculated from pre (t) and post ($t+1$) growing season measurements of girth (G cm) as $\ln(G^3_{t+1}) - \ln(G^3_t)$. Significance tests were based on separate 2-way analysis of variance for each year, with two levels of insecticide treatment (+ or 0) and three size classes of trees ($G<25$, $25 \leq G<50$, $G \geq 50$ cm); NS, not significantly different. Defoliation was caused principally by *Tortrix viridana* and *Operophtera brumata* but with many less important Lepidoptera. Peduncles measured in August include fruit stalks with acorns, galls, undeveloped fruits and unpollinated flower remains; not all peduncles bear sound acorns. Galls refer to the 'knopper galls' of *Andricus quercuscalicis*

The results of the experiment described here are consistent with the hypothesis that oak is essentially a K-strategist[19,20], gaining in fitness by first ensuring its survival and by putting its carbohydrate and protein resources into seed production only when the needs of reserve-replenishment, wood growth, root growth and bud-filling have been met. Although several other studies of natural[21,22] and artificial defoliation of trees[23,24] have shown reduced seed production, these have involved typically much higher rates of defoliation (50–100%). The present study is the first to show that natural low levels of insect feeding can cause a significant reduction in seed set. As the number of female flowers produced in spring does not differ between the two treatments in most years, the increase in acorn production is seen to result from a reduction in the number of peduncles aborted and an increase in the average number of acorns filled on each surviving peduncle. There is no evidence to suggest that insects feeding directly on the flowers or young acorns contribute to the observed result.

As there is no simple relationship between seed production in one year and seedling recruitment in the next[9], we cannot be sure that these low levels of defoliation actually affect plant population density. This study does emphasize, however, that it is incorrect to assume that 'typical' low density populations of defoliating and sucking herbivores can have no impact on the demography of the tress. Also, given the great variation from tree to tree in average defoliation levels and the magnitude of the differences in fecundity observed in this experiment, these relatively scarce herbivores may act as potent agents of natural selection.

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Received 15 October 1984, accepted 7 January 1985

1 Bray, J R *Ecology* **45**, 165–167 (1964)
2 Golley, F, Odum, H T & Wilson, R F *Ecology* **43**, 9–19 (1962)
3 Haukioja, E *Tundra Ecosystems A Comparative Analysis* (eds Bliss, L C, Heal, O W. & Moore, J J) 547–555 (Cambridge University Press, 1981)
4 Kaczmarek, W *Secondary Productivity of Terrestrial Ecosystems* (ed Petrusewicz, K) 663–678 (IBP, Warsaw, 1967)
5 Reichle, D E, Goldstein, R A, Van Hook, R I & Dodson, G J *Ecology* **54**, 1076–1084 (1973)
6 Rothacher, J S, Blow, F E & Potts, S M *J. For* **52**, 169–173 (1954)
7 Gradwell, G *The British Oak* (eds Morris, M G & Perring, F H) 182–193 (Classey, Farringdon, 1974)
8 Jameson, D A *Bot Rev* **29**, 532–594 (1963)
9 Crawley, M J *Herbivory* (Blackwell Scientific, Oxford, 1983)
10 Heichel, G H & Turner, N C *Oecologia (Berlin)* **57**, 14–19 (1983)
11 Varley, G C & Gradwell, G R *Proc. 11th int Congr Ent* **2**, 211–214 (1962)
12 May, R M *Theoretical Ecology* 2nd edn (Blackwell Scientific, Oxford, 1981)
13 Price, P W *et al A Rev ecol. Syst* **11**, 41–65 (1980)
14 Morrow, P A & LaMarche, V C *Science* **210**, 1224–1226 (1978)
15 Stevens, G R *Conn agric. exp Stn Bull* **796**, 1–13 (1981)
16 Worthing, C R *The Pesticide Manual* 7th edn (British Crop Protection Council, London, 1983)
17 Southwood, T R E *Ecological Methods* 2nd edn (Chapman & Hall, London, 1976)
18 Collins, M, Crawley, M J & McGavin, G C *Ecol Ent* **8**, 133–138 (1983)
19 Caswell, H *Ecology* **63**, 1218–1222 (1982)
20 Stearns, S C. *Oikos* **35**, 266–281 (1980)
21 Torrent, J. A *FAO Pl Prot Bull* **3**, 117–121 (1955)
22 Kulman, H M *A Rev Ent* **16**, 289–324 (1971)
23 Rockwood, L L *Ecology* **54**, 1363–1369 (1973)
24 Heichel, G H & Turner, N C *Oecologia (Berlin)* **62**, 1–6 (1984)

Firing patterns of motor units in normal rats

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Skeletal muscles consist of motor units which may differ considerably in contractile properties and types of usage. Some units participate mainly in relatively rare, quick movements and contract rapidly and are easily fatigued (type FF); others contribute to the maintenance of posture and hence contract slowly and are fatigue-resistant (type S), while others are both fast and fatigue-resistant (type FR; ref. 1). Our understanding of motor control mechanisms and the dependence of contractile properties on usage would be enhanced if more quantitative information were available[2–4] concerning the firing patterns of individual motor units during normal motor behaviour. Therefore, we have made continuous recordings for extended periods from single motor units in the fast extensor digitorum longus (edl) and the slow soleus (sol) muscle of freely moving adult rats. By counting the total number of discharges for each unit, and by determining the distributions of interspike intervals and the duration of the individual impulse trains, we have obtained information about firing rate, amount of use, modulation of muscle force and tonic and phasic behaviour for 16 motor units. We now report that these units fall into three classes apparently corresponding to type FF and FR in the edl muscle and type S in the soleus muscle.

Figure 1*a–c* shows representative discharges from two units in edl (*a* and *b*) muscle and one unit in soleus (*c*) muscle. The unit potentials were similar in shape and amplitude throughout the 24-h recording, and superimposing the traces indicated that they originated from one unit only (Fig. 1*d–f*). In Table 1 the units have been ordered according to the percentage of total time that they were active. Three classes of firing patterns can be discerned. In the first class (edl-1 units) the units were active for only 0.5–3 min per 24 h (0.04–0.22% of the total time). In the second class (edl-2 units) the total active time was 23–72 min (1.6–5%), and in the third class (sol-1 units), 5.3–8.4 h (22–35%). The soleus units formed a separate class by firing more impulses at a lower frequency (~20 Hz) than any edl units. The two classes of edl units could not be clearly distinguished on the basis of firing rate alone, although, on the whole, edl-1 units fired at higher frequencies than edl-2 units (Fig. 2, Table 1). The edl-1 units, however, were by far the least active units, generating impulse trains that were always brief and in 70–80% of cases started with a pair of spikes having a short interspike interval (initial doublet of Fig. 1*a*, *d*). In some respects edl-2 units occupied an intermediate position in that they resembled edl-1 units by firing at high frequency and sol-1 units by generating a large number of impulses which sometimes occurred as long trains of impulses.

The firing pattern of soleus units was relatively uniform. There was some variation between units in amount of activity, but little variation in median firing rates. This pattern probably represents S-type motoneurones innervating slow, fatigue-resistant type I muscle fibres which make up the large majority of fibres in adult rat soleus muscle[5]. The edl muscle contains almost exclusively fast motor units[6]. When stained for myosin ATPase activity, approximately half the muscle fibres appear as type IIA and the other half as type IIB[7], which, in general, correspond to FR and FF units, respectively[1]. It seems likely, therefore, that the infrequently used edl-1 units represent FF units, whereas the much more frequently used edl-2 units correspond to FR units.

There exists evidence that motor unit characteristics are more continuously distributed than is suggested by Table 1 (refs 1, 8). The clear separation between soleus and edl units is not surprising given such widely differing muscles. Within the edl, however, a larger unit sample would probably have narrowed the gap between the two classes of firing patterns, and the existence of intermediate types cannot be excluded. Nevertheless, even within the three classes of firing patterns presented in Table 1, there is considerable variation in the number of discharges and in the frequency of spikes within each discharge—this variation may partly explain the variation in contraction speed and metabolic enzyme activity observed in different motor unit types and in type I, IIA and IIB fibre populations[1,9,10].

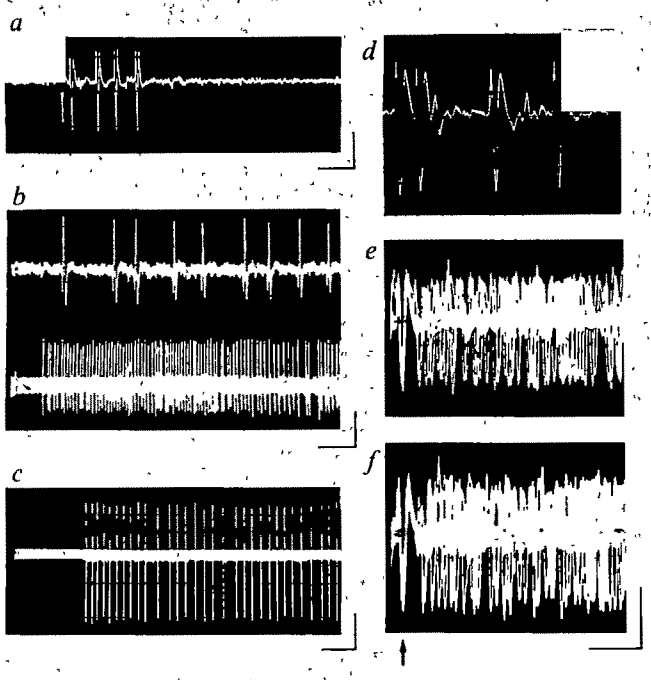


Fig. 1 Typical discharges for each of the three classes of motor units shown in Table 1: *a*, edl-1 unit; *b*, edl-2 unit; *c*, sol-1 unit. Traces *d*–*f* show that it is possible to record continuously for 24 h from one motor unit (in this case an edl-1 unit). Spontaneous action potentials trigger the oscilloscope trace and are displayed after a fixed delay (arrow) on a single trace (*d*) or on ~100 superimposed traces (*e* and *f*). The action potentials have a similar appearance at the beginning (*e*) and at the end (*f*) of the 24-h recording. Note that there is also a minimum spike interval after the first triggered spike in each of the traces in *e* and *f*, which makes the presence of more than one unit highly unlikely. Vertical bars represent 0.5 mV and horizontal bars 20 ms (*a*, *b*), 200 ms (*b*, *c*) and 10 ms (*d*–*f*). The motor unit potentials were similar in amplitude (0.5–1 mV) to fibrillatory potentials recorded from the same electrodes 2–3 days after cutting the sciatic nerve, indicating that the potentials were generated by single muscle fibres close to the recording electrodes.

Methods. Adult male Wistar rats were anaesthetized with chloral hydrate and pentobarbital (Equithesin, 0.4 ml per 100 g). Four Teflon-coated platinum wires (outer diameter 0.25–0.50 μm), soldered to the ends of four flexible Teflon-coated multi-stranded steel wires, were guided by a thin needle and tube longitudinally into the centre portion of the edl or sol muscle. The distance between each cut end was ~100 μm. The steel wires continued under the skin, through an attachment fixed to the skull by screws and dental cement, into a flexible protective tube which connected the rat to rotating contacts and preamplifiers above it. Muscle action potentials were recorded between the ends of the two wires that gave the best spike isolation and signal-to-noise ratio. The action potentials were stored on tape, and reproduced later on a storage oscilloscope for examination of the entire 24-h recording. Only potentials that could be attributed confidently to the same unit for the entire 24-h period were accepted for further computer analysis. The recordings were usually best 2–3 days after implantation; thereafter, the potentials often declined in amplitude, but with occasional improvements. Such changes made it difficult to be sure that we were recording from the same unit for periods much longer than 24 h. From the time of implantation the rats were kept in the laboratory in large buckets where they moved freely, were more active during the night and showed apparently normal behaviour in terms of movement, feeding and sleeping.

If we are justified in assigning the firing patterns of edl-1, edl-2 and sol-1 units to FF, FR and S-type motor units, some interesting relations between firing pattern and contractile properties appear. First, low firing frequency characterizes slow motor units, whereas high firing frequency characterizes fast motor units. Second, motor units that are used frequently are more fatigue-resistant than those used infrequently. Third, motoneurones fire naturally at frequencies that give maximum control over the tension developed by the muscle fibres. Figure

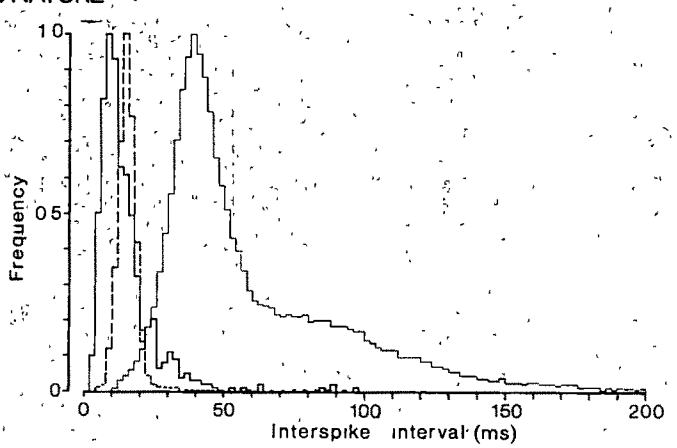


Fig. 2 Normalized frequency distribution of the interspike intervals obtained during a 24-h recording from the units shown in Fig. 1*a*–*c*. The thick solid line shows the distribution for the edl-1 unit; broken line, the edl-2 unit; and the thin continuous line, the sol-1 unit. The histograms contain, respectively, 686, 1,223 and 91,097 intervals and are typical of the three classes of motor units shown in Table 1. Intervals longer than 200 ms, representing only a very small fraction of the total, have been omitted. From such histograms, an impulse train was defined as starting (and stopping) when the duration of the preceding (and subsequent) intervals was at least 200 ms for edl units and 500 ms for sol units; this definition ensured that the tension produced by any preceding impulse declined to zero before another train was said to start.

3*a* shows the tension developed by stimulating soleus and edl muscles at different frequencies. In soleus muscle twitches begin to fuse at ~8 Hz and maximum tetanus is reached at ~100 Hz. In edl, similar force summation occurs between 30 and 200 Hz. The natural firing rates of soleus and edl units (Fig. 3*b*) show a striking correspondence to the rising portions of their respective tension–frequency curves (Fig. 3*a*), which cover the frequencies over which muscle force can be effectively modulated.

These relationships support recent evidence that the pattern of evoked muscle activity has an important role in the regulation of speed of contraction and fatigue resistance[11,12]. Thus, a large amount of low-frequency (10 Hz) stimulation of the nerve induces both slow contraction speed and high fatigue resistance in fast muscles[13,14]. Conversely, the slow soleus muscle contracts much faster when denervated and stimulated at high frequency (ref. 15 but see also ref. 11) and becomes less fatigue-resistant if the amount of stimulation is kept low[16]. Similar, but less pronounced, speeding up occurs if the nerve to the soleus is intact and the muscle continues to show tonic low-frequency activity in between the high-frequency stimulus trains (T.L. and R.H., unpublished). Mechanisms appear to exist, therefore, whereby the contractile properties of muscle fibres can be adjusted continually to the pattern of afferent impulse activity. Thus, effective performance can be maintained throughout life despite changes in the level and pattern of activity.

Certain features of the edl-1 firing pattern deserve special mention. First, edl-1 units rarely generated more than five or six impulses at a time: a single impulse was by far the most common event, two impulses per burst the next common event and so on. By varying the number of stimuli from 1 to 5 in a train of stimuli to the edl muscle, we found that the force output can be varied between ~15% and 80% of maximal tetanic tension. Hence, the force output of the motor unit must normally show considerable variation as a result of the observed variation in number of motoneurone discharges per burst. Second, although the total number of impulses in edl-1 units was 1–2 orders of magnitude lower than in edl-2 or sol-1 units, the edl-1 units nevertheless discharged trains of impulses on hundreds or thousands of occasions per day. Assuming that the edl-1 units are FF units, this suggests that FF units participate not only in relatively rare, high-force quick movements such as jumping,

Table 1 Firing patterns of individual motor units in the rat

Motor unit	Amount of activity		Burst characteristics			
	% Time occupied by bursts	No. of impulses per day	First interval (Hz)	All intervals (Hz)	No. of impulses per burst	Longest duration (s)
edl-1	0.04	2,600	250 (333–167)	91 (111–58)	3 (1–18)	0.83
	0.07	4,600	167 (333–125)	83 (111–67)	3 (2–16)	1.15
	0.09	5,600	143 (200–77)	77 (91–67)	13 (3–35)	3.9
	0.14	7,800	125 (200–77)	77 (83–53)	5 (2–12)	1.1
	0.22	11,200	91 (167–63)	67 (77–50)	4 (1–8)	1.15
edl-2	1.6	105,500	71 (125–46)	83 (100–67)	3 (1–11)	59
	2.0	89,500	50 (100–40)	53 (59–50)	25 (3–65)	120
	2.6	119,200	43 (56–30)	56 (67–40)	6 (2–18)	141
	3.5	139,400	36 (44–26)	48 (53–42)	4 (2–13)	89
	5.0	243,100	41 (67–29)	58 (67–53)	39 (6–56)	135
sol-1	22	309,500	13 (23–7)	21 (26–12)	5 (1–35)	300
	29	449,500	14 (24–6)	19 (26–14)	8 (1–39)	390
	31	453,400	14 (29–6)	19 (29–14)	7 (1–38)	548
	31	446,400	14 (25–6)	19 (23–13)	9 (1–35)	290
	32	482,500	15 (31–5)	21 (26–15)	10 (1–40)	510
	35	495,800	14 (23–6)	18 (25–13)	7 (1–36)	490

Columns 4 and 5 give median values for the instantaneous frequencies for certain intervals in all the bursts generated by one motor unit in one day. Column 4 refers to the first interval and column 5 to all intervals in each burst. Note that only in the edl-1 units is the frequency highest at the onset of a burst, in agreement with the frequent occurrence of initial doublets in these units (Fig. 1*a*, *d*). Numbers in parentheses are quartile values.

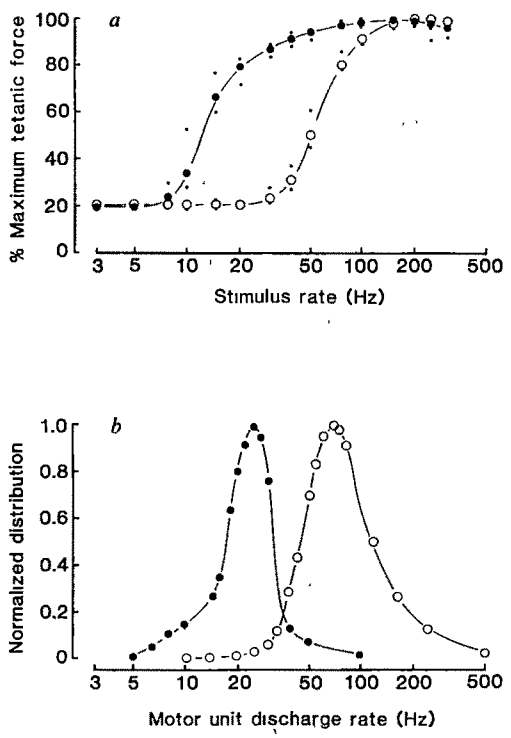


Fig. 3 Correspondence between tension-frequency curves (*a*) and motor unit firing rate (*b*) in soleus (●) and edl (○) muscles. The tension-frequency curves were obtained in acute experiments *in situ*, at 35 °C and at optimal muscle length by stimulating supramaximally the nerves to normal soleus and edl muscles. Each symbol represents the median isometric tension obtained from eight (sol) or six (edl) muscles and represents the highest tension produced regardless of when this occurred during a 1-s stimulus train at the frequencies indicated. Total ranges that extend beyond the symbols are indicated by dots. The curves in *b* were obtained from histograms of the type shown in Fig. 2 for all the units shown in Table 1. Each histogram was divided into segments of 5-ms duration, and the number of interspike intervals within each segment were counted, converted to frequency by taking the reciprocal values and expressed relative to the most commonly occurring frequency for all 6 units in soleus muscle and 10 units in edl muscle.

but also in more frequent, brief, inconspicuous contractions, possibly of a corrective nature, such as those described for high threshold units in humans[4]. The total amount of activity in edl-1 units, expressed as average firing rate over ⩾1 day, was only 0.03–0.13 Hz (Table 1). Even lower mean impulse rates prevent supersensitivity to acetylcholine and other abnormal responses in denervated muscles if the activity is evoked relatively often by direct stimulation, for example, by a brief 100-Hz stimulus train applied every 2 h (mean frequency 0.01 Hz; refs 16, 17 and unpublished observations). In contrast, denervated organ-cultured muscles, which fibrillate relatively intensely for ~20 h, then remain silent for 2–3 days before a new cycle is repeated, show much higher average firing rates (~1 Hz) and yet become both atrophic and supersensitive to acetylcholine[18]. This suggests that timing, rather than the amount of activity, is important for the maintenance of normal muscle function. The relatively frequent activation of edl-1 units may explain, therefore, why FF units retain normal properties despite high activation thresholds[19] and low total amounts of activity.

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1 Burke, R. E *Handbook of Physiology* Sect I, Vol 2, Pt 1, 345–422 (American Physiological Society, Bethesda, Maryland, 1981).
2. Fischbach, G D & Robbins, N *J Physiol., Lond* **201**, 305–320 (1969).
3 Navarrete, R. & Vrbová, G *J Physiol., Lond.* **351**, 675–685 (1984)
4 Grimby, L *J Physiol., Lond.* **346**, 195–202 (1984)
5 Kugelberg, E *J neurol. Sci.* **27**, 269–289 (1976)
6 Close, R. *J Physiol., Lond.* **193**, 45–55 (1967)
7 Niederle, B. & Mayr, R. *Anat Embryol.* **153**, 9–21 (1978)
8 Kernell, D in *Brain and Spinal Mechanisms of Movement* (ed Desmedt, J. E.) 213–226 (Raven, New York, 1983)
9 Kugelberg, E & Lindegren, B *J Physiol., Lond.* **288**, 285–300 (1979).
10 Reichmann, H & Pette, D. *Histochemistry* **74**, 27–41 (1982)
11 Jolesz, F & Sreter, F A. *A. Rev Physiol.* **43**, 531–552 (1981).
12 Salmons, S. & Henriksson, J *Muscle Nerve* **4**, 94–105 (1981).
13 Pette, D , Smith, M. E , Staudte, H W & Vrbová, G *Pflügers Arch. ges Physiol.* **338**, 257–272 (1973).
14 Salmons, S & Sreter, F A. *Nature* **263**, 30–34 (1976)
15 Lømo, T , Westgaard, R. H. & Dahl, H A *Proc. R. Soc.* **B187**, 99–103 (1974).
16. Lømo, T., Westgaard, R. H & Engebretsen, L in *Plasticity of Muscle* (ed Pette, D) 297–309 (Walter de Gruyter, New York, 1980)
17 Lømo, T. & Westgaard, R. H. *J Physiol., Lond.* **252**, 603–626 (1975).
18 Purves, D. & Sakmann, B *J Physiol., Lond.* **237**, 157–182 (1974)
19 Kernell, D & Monster, A W *Brain Res* **229**, 193–196 (1981)

Stimulation of food intake in rats by centrally administered hypothalamic growth hormone-releasing factor

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Hypothalamic growth hormone-releasing factors (GRFs) have been purified recently from human pancreatic (hp) tumours[1,2] and from rat hypothalamus (rh)[3]. GRF peptides have strong homology with peptides of the glucagon, vasoactive intestinal polypeptide and PHI-27 family. Aside from their potent actions on release of somatotropin, no other biological actions of GRFs have been reported. GRF has been localized in neurones bordering the ventromedial hypothalamic nucleus (refs 4, 5; L. Swanson and P. Sawchenko, manuscript in preparation), a region associated frequently with experimental analysis of feeding behaviour[6]. We now report that intracerebroventricularly (i.c.v.)-administered rhGRF and hpGRF(1–40) in doses of 0.2, 2.0 and 20.0 pmol, produced an increase in food intake in hungry rats. This effect seemed to be specific to GRF as i.c.v. injections of a structurally related but physiologically inactive peptide[7] in the same doses had no effect on feeding. In addition, peripheral injections of rhGRF or growth hormone had no effect on food intake, suggesting that the present effects may be mediated centrally. Injections (i.c.v.) of rhGRF (0.2, 2.0 and 20.0 pmol) had no effect on general activity, suggesting that GRF does not produce nonspecific arousal.

Thirty-two male Wistar rats (250–300 g) were used and a 23-gauge guide cannula was implanted 1 mm dorsal to the right or left lateral ventricle of each rat (coordinates for the surgery using the Pellegrino and Cushman atlas[8]: 0.6 mm posterior to bregma, 2.0 mm lateral of the midline suture, 3.2 mm ventral of the dorsal surface of the skull). Rats were then returned to their home cages where they had free access to food and water and were allowed 1 week to recover from surgery. On the eighth day after surgery (day 1), rats were started on a food deprivation schedule consisting of depriving the rats of food for 23 h and then placing them in individual wire testing chambers (20 × 25 × 36 cm) where they had access to wet mash [ratio of water/food 7:5 (v/v)] for 30 min. Food intake during this period was determined by calculating the difference between pre- and post-test food weight (including spillage). Rats were then returned to their home cages and allowed a further 30 min access to their regular laboratory diet after which the food was removed, but water was still available throughout the 23 h of food deprivation. This procedure was repeated for 5 days; by day 5, rats had stabilized their food intake during the 30-min test session (± 10% of average of the last 2 days). On day 6, all rats were pretreated 30 min before the feeding test session with one of four doses of rhGRF (0.0, 0.2, 2.0 or 20.0 pmol) administered i.c.v. All injections were made in a 2-μl volume of 0.01% ascorbic acid with a 30-gauge injector using gravitational force. Rats were maintained on the deprivation schedule following the day 6 test session and again tested for food intake the following day. In a second experiment, 26 different rats were tested with hpGRF(1–40) for food intake using the same method.

Both rhGRF and hpGRF(1–40) had a stimulatory effect on food intake. Figure 1 shows that although there was little change in the amount of food intake in rats treated with vehicle alone, there was an increase in intake for hpGRF(1–40)- and rhGRF-treated rats. A one-way analysis of variance revealed that this effect is statistically significant for both the hpGRF(1–40)-treated rats ($F(3, 22) = 4.398$, $P = 0.014$) and the rhGRF-treated rats ($F(3, 28) = 3.159$, $P = 0.039$). (All individual group comparisons were carried out using the Duncan multiple range *a posteriori* test.) Individual group comparisons with vehicle-treated rats revealed significant increases in food intake at 0.2, 2.0 and 20.0 pmol for both the hpGRF(1–40)-treated rats ($P < 0.05$) and the rhGRF-treated rats ($P < 0.05$). The mean food intake (±s.e.m.) on control days was 21.3 ± 2.1 g for rhGRF-treated rats and 28.5 ± 2.2 g for hpGRF(1–40)-treated rats. Both hpGRF(1–40)- and rhGRF-treated rats returned to baseline levels of food intake on the day after GRF tests: $F(3, 22) = 0.387$, not significant (NS) and $F(3, 28) = 0.720$, NS, respectively.

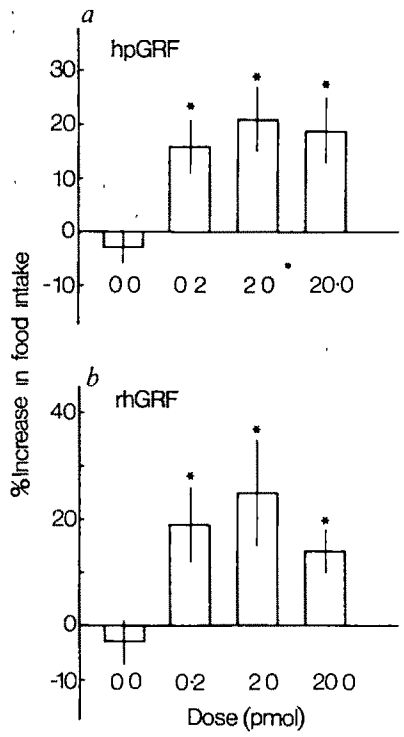


Fig. 1 The mean per cent increase in food intake after i.c.v. injections of *a*, hpGRF(1–40) ($n = 7$ for vehicle and 2.0-pmol groups, $n = 6$ for 0.2- and 20.0-pmol groups) and *b*, rhGRF ($n = 8$ for each group). 0.2, 2.0 and 20.0 pmol correspond to 1.0, 10.0 and 100.0 ng, respectively. Rats were assigned to their respective drug condition on the basis of their food intake on the previous 2 days such that the mean food intake before GRF treatment was approximately the same for all four drug treatment groups. Error bars represent the s.e.m. * Significantly different from vehicle-treated group, $P < 0.05$, Duncan multiple range *a posteriori* test.

In a third experiment using the same procedure, 24 rats were tested with GRF(3–40), a structurally related peptide completely unable to stimulate growth-hormone release[7], as a control for possible nonspecific effects of i.c.v. GRF injections. There were no effects of GRF(3–40) on food intake ($F(3, 20) = 1.007$, NS), suggesting that the facilitation in food intake observed in experiments 1 and 2 was probably not due to nonspecific effects of i.c.v. peptide administration.

Rats were maintained on the deprivation schedule following GRF(3–40) testing to investigate whether the increased food intake reflected an increased release of adenohypophyseal growth hormone or a direct result of peripheral responses that might also affect feeding. Thus, the same rats used for GRF(3–40) tests were re-divided into three groups and tested with rhGRF (0, 2.0 or 200.0 pmol) on day 8 by intraperitoneal (i.p.) injection in a 0.4-ml volume of 0.01% ascorbic acid. The peripheral injections of rhGRF had no significant effect on food intake ($F(2, 21) = 0.637$, NS). An additional six rats were subsequently tested with 0.2 pmol rhGRF injected i.p. Consistent

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with the effects of 2 and 200 pmol, 0.2 pmol of rhGRF injected i.p. had no significant effect on food intake, $F(5) = 0.7$, NS. The fact that peripheral rhGRF did not influence food intake suggests that the GRF-induced facilitation of feeding is due neither to increased growth-hormone release nor to actions of GRF directly in the periphery.

To examine further the possibility that the present facilitatory effects of GRF on feeding are due to peripheral actions of GRF or increased growth-hormone release, the effects of peripheral injections of rat growth hormone were tested by the same procedure as experiments 1 and 2. On day 6, 24 rats were pretreated 30 min before the feeding session with one of four doses of rat growth hormone (0, 10, 50 and 250 μg per rat) administered i.p. These doses were chosen based on findings indicating that growth hormone values may normally reach 2 μg ml^{-1} in rat blood following endogenous GRF secretion (W.V., unpublished data). All injections were made in 0.4 ml of 0.03 M $NaHCO_3$ in 0.15 M NaCl and six rats were assigned to each growth hormone dose.

Our results demonstrated that rat growth hormone had no significant effect on food intake ($F(3, 20) = 0.313$, NS). The mean per cent control food intake (±s.e.m.) after 0-, 10-, 50- and 250-μg treatments was 104±10, 98±2, 100±7 and 95±3, respectively. The mean food intake (±s.e.m.) on control days was 25.8±1.1 g, suggesting that in the present conditions, the GRF-induced facilitation in food intake was probably not due to an increase in growth hormone release.

We tested the hypothesis that the increases in feeding observed following i.c.v. GRF treatment are the result of the general behavioural activating properties of GRF. Thus, 24 rats were tested for their locomotor response to one of the following doses of rhGRF: 0.0, 0.2, 2.0 or 20.0 pmol. rhGRF was administered i.c.v. as in experiment 1 and six rats were tested with each dose. Locomotor activity was measured as in previous studies[9]. We found no significant group effect of rhGRF on locomotor activity ($F(3, 20) = 0.399$, NS). Mean photocell beam interruptions (± s.e.m.) for the 0-, 0.2-, 2.0- and 20.0-pmol doses of rhGRF were 334±59, 438±87, 260±50 and 392±94, respectively. These results suggest that rhGRF, in the doses which produced a stimulation in food intake in the present study, do not produce general behavioural activation.

Our finding that low doses of centrally injected GRF can stimulate feeding is the first demonstration of a behavioural role for GRF. It is not yet clear whether the appetitive behaviour promoted by GRF is involved functionally in other peripheral actions initiated by central GRF secretion. Thus, the classic effects of growth hormone to promote growth of soft tissue in bone and cartilage[10], together with the central appetitive effect of GRF, suggest that central and peripheral factors may collectively underly growth processes in an integrated manner. Citing the actions of luteinizing hormone-releasing hormone, Moss and McCann[11] have proposed previously the concept of a central/peripheral integration of neuropeptide function. In the present study we add the actions of GRF as another possible case in point.

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1. Rivier, J., Spiess, J., Thorner, M & Vale, W *Nature* **300**, 276-278 (1982).
2. Guillemin, R *et al. Science* **218**, 585-587 (1982)
3. Spiess, J., Rivier, J & Vale, W *Nature* **303**, 532-535 (1983)
4. Bloch, B., Gaillard, R. C., Brazeau, P., Lin, M D. & Ling, N. *Regulatory Peptides* **8**, 21-31 (1984).
5. Bloch, B. *et al. Nature* **263**, 251-257 (1983)
6. Carlson, N. R in *Physiology and Behavior*, 355-363 (Allyn & Bacon, Boston, 1977)
7. Rivier, J., Spiess, J & Vale, W. in *Proc. 8th Am. Peptide Symp* (eds Hruby, V J & Rich, D D.) 853-856 (Pierce Chemical Co., Rockford, 1983)
8. Pellegrino, L J., Pellegrino, A S & Cushman, A J *A Stereotaxic Atlas of the Rat Brain* (Plenum, New York, 1979)
9. Joyce, E M. & Koob, G. F. *Psychopharmacology* **73**, 311-313 (1981)
10. Lee, J. & Laycock, J. *Essential Endocrinology* (Oxford University Press, 1978)
11. Moss, R. L & McCann, S M *Science* **181**, 177-179 (1973)

Co-localization of $GABA_A$ receptors and benzodiazepine receptors in the brain shown by monoclonal antibodies

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The most abundant inhibitory neurotransmitter in the central nervous system, γ-aminobutyric acid (GABA), exerts its main effects via a $GABA_A$ receptor that gates a chloride channel in the subsynaptic membrane[1]. These receptors can contain a modulatory unit, the benzodiazepine receptor, through which ligands of different chemical classes can increase or decrease $GABA_A$ receptor function[2,3]. We have now visualized a $GABA_A$ receptor in mammalian brain using monoclonal antibodies. The protein complex recognized by the antibodies contained high- and low-affinity binding sites for GABA as well as binding sites for benzodiazepines, indicative of a $GABA_A$ receptor functionally associated with benzodiazepine receptors. As the pattern of brain immunoreactivity corresponds to the autoradiographical distribution of benzodiazepine binding sites, most benzodiazepine receptors seem to be part of $GABA_A$ receptors. Two constituent proteins were identified immunologically. Because the monoclonal antibodies cross-react with human brain, they provide a means for elucidating those CNS disorders which may be linked to a dysfunction of a $GABA_A$ receptor.

Monoclonal antibodies were raised in mice immunized with a highly purified $GABA_A$ receptor/benzodiazepine receptor fraction from bovine cerebral cortex[4], a preparation characterized by: (1) the presence of binding sites for benzodiazepines as tested by ^{3}H-flunitrazepam (^{3}H-FNZ) binding; (2) high-affinity binding sites for GABA, assayed by ^{3}H-muscimol binding; and (3) low-affinity binding sites for GABA, monitored by the allosteric enhancement of benzodiazepine receptor affinity in micromolar concentrations of GABA[4]. The ligand specificity of these binding sites corresponds to that of the $GABA_A$ receptor/benzodiazepine receptor complex (receptor complex) in synaptic membranes[5]. The presence of low-affinity GABA binding sites coupled to benzodiazepine receptors is of interest as, electrophysiologically, the receptor complex is activated by micromolar concentrations of GABA[6-10]. We obtained a total of 22 monoclonal antibodies, with which 4 different epitopes of the $GABA_A$ receptor complex could be distinguished. We describe here two antibodies (bd-17 and bd-24) representative of the interaction with two of the receptor epitopes.

Antibodies recognizing the receptor complex would be expected to immunoprecipitate with a compound having the binding characteristics of the purified receptor. To test this premise, a bovine receptor complex fraction[4] was incubated with the antibody bd-17 or bd-24, followed by precipitation with goat/anti-mouse immunosorbent. As neither antibody inhibits the binding of ^{3}H-FNZ or ^{3}H-muscimol, the resuspended immunosorbent beads were tested in radioligand binding assays (Fig. 1). In controls without bd-17 or bd-24, no binding of ^{3}H-FNZ or ^{3}H-muscimol was found. When increasing concentrations of either bd-17 or bd-24 were added, however, increasing amounts of the binding sites for ^{3}H-FNZ and ^{3}H-muscimol were recovered, ≤90–95% of the total activity (Fig. 1). Binding of the benzodiazepine ^{3}H-diazepam (5 nM, 90 min, 4 °C) to the pellets was increased by 50±5% when tested in the presence of 10 μM GABA, indicating the presence of low-affinity GABA sites. The ligand specificity of these sites corresponds to that of membrane-bound $GABA_A$ receptors as benzodiazepine receptor binding was also enhanced by muscimol (1 μM), 3-aminopropanesulphonic acid and tetrahydroisoxazolopyridinol (THIP, 10 μM), but not by the $GABA_B$ receptor agonist

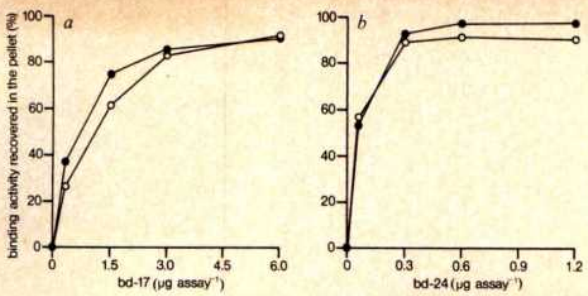


Fig. 1 Immunoprecipitation of binding sites for benzodiazepines and GABA with two monoclonal antibodies (*a*, bd-17; *b*, bd-24), recognizing different receptor epitopes. Filled and open circles represent specifically bound ^{3}H-FNZ and ^{3}H-muscimol, respectively

Methods. A partially purified $GABA_A$ receptor/benzodiazepine receptor preparation from bovine cerebral cortex (100 μl, ~30 μg ml^{-1}; ref. 4) was incubated for 2 h at 4 °C with 200 μl of bd-17 or bd-24 (previously purified by affinity chromatography on a sheep/anti-mouse antibody column), respectively. 200 μl immunosorbent (goat anti-mouse immunoglobulin G-agarose, Sigma A-6531, in phosphate-buffered saline (PBS), 0·2% Triton X-100 at a v/v ratio of 1:1) were added and the incubation continued for another 2½ h in the presence of 1% bovine serum albumin (BSA). After centrifugation, the pellet was washed once in 1 ml cold PBS, 0.2% Triton X-100, 1% BSA. The first supernatant and the final pellet were subjected to separate radioligand binding assays with ^{3}H-FNZ (10 nM, 60 min) and ^{3}H-muscimol (100 nM, 30 min). The radioligand binding assay for the supernatant was as described previously[21]. Radioligand binding to the pellet was performed in an Eppendorf tube on a turning wheel at 4 °C in 1 ml (final volume) of assay buffer (50 mM Tris/citrate *p*H 7.1 + 0.2% Triton X-100) containing the radioligand. After a short centrifugation, the resuspended pellet and rinse fluid of the tube were transferred onto a glass fibre filter and washed with 2 × 5 ml cold buffer. The radioactivity retained on the filter was measured using an external standard procedure. For each ^{3}H-FNZ and ^{3}H-muscimol binding experiment, nonspecific binding was determined in a parallel assay in the presence of 3 μM diazepam or 1 mM GABA, respectively. In controls which contained either no or an irrelevant antibody, only 7% of the binding activity was recovered in the pellet. This nonspecific precipitation was subtracted from all values. The results are expressed as % of total activity (100% = 100,000 d.p.m. ^{3}H-FNZ and 44,000 d.p.m. ^{3}H-muscimol, respectively): mean values of two experiments which differed <10% are given. Based on antibody concentration, the antigen-binding efficiency of bd-24 was about 10-fold higher than that of bd-17.

baclofen (100 μM), Thus, the immunoprecipitated material contained binding sites for benzodiazepines as well as low- and high-affinity binding sites for GABA, indicating that the antibody recognized the receptor complex. The same results were obtained with a purified receptor preparation from postmortem human cerebral cortex.

The presence of benzodiazepine receptors in the immunoprecipitated pellet was confirmed further by identifying the receptor protein with a covalently bound radioligand. When a purified bovine receptor sample was photoaffinity labelled with ^{3}H-FNZ (10 nM, 5 min ultraviolet; ref. 11) and immunoprecipitated subsequently with saturating amounts of either bd-17 or bd-24, 90% of the radioactivity incorporated into the protein was recovered in the pellet.

Benzodiazepine receptors in rat brain membranes consist of subunits of relative molecular mass (M_r) 50,000 and 55,000 (refs 11, 12). Similarly, a highly purified receptor complex from bovine brain contains two subunits of M_r 50,000–53,000 and 55,000–57,000, respectively[4,13–15]. We therefore expected these proteins to be recognized by the antibodies bd-17 and bd-24 in immunoblot experiments. In receptor preparations from postmortem human or bovine cerebral cortex, bd-24 labelled specifically a protein of M_r 50,000 and bd-17 a protein of M_r 55,000 (Fig. 2). Thus, each antibody apparently interacted with only one of the two putative constituent proteins of the receptor complex (Fig. 2). Both antibodies, however, were able to

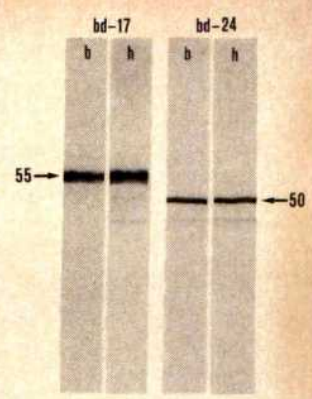


Fig. 2 Immunoblot of a bovine (b) and human (h) receptor complex preparation, showing the specificity of bd-17 and bd-24 for proteins of M_r 55,000 and 50,000, respectively. **Methods.** Partially purified receptor complex preparations from bovine or human cerebral cortex[4] were subjected to SDS-polyacrylamide gel electrophoresis. The resolved polypeptides were electroblotted onto a nitrocellulose membrane (HAWP, 0.45 μm pore size, Millipore) at 1 A for 90 min at room temperature in 25 mM Tris, 192 mM glycine and 20% (v/v) methanol, *p*H 8.3, according to Towbin *et al.*[22]. Unreacted sites on the nitrocellulose were blocked by three 10-min washes in 0.05% Tween-20 in PBS (PBS-Tween), according to De Blas and Cherwinski[23]. Strips of nitrocellulose were then incubated in bd-17 or bd-24 hybridoma supernatants, containing 0.05% Tween-20, at room temperature overnight under rocking. After three 10-min washes with PBS-Tween, the strips were incubated with ^{125}I-labelled sheep anti-mouse immunoglobulin (0.5–1.0 × 10^6 c.p.m. ml^{-1}; 30–60 ng ml^{-1}) in PBS-Tween, for 3 h at room temperature under rocking. Affinity purification and iodination of sheep anti-mouse immunoglobulins has been described previously[24]. Membrane strips were then washed 4× for 20 min in PBS-Tween and once in H_2O, dried and exposed to Kodak X-Omat AR film, using an intensifying screen (DuPont). M_r values of 50,000 and 55,000 are indicated by 50 and 55 respectively. The immunoreactive minor bands were presumably degradation products. All postmortem human brain was obtained through the Institutes of Pathology, Kantonsspital, Liestal and Basel (Switzerland).

immunoprecipitate the entire receptor population (Fig. 1), which suggests that the receptor complex has a mixed subunit composition. Furthermore, the immunoblot pattern (Fig. 2) excludes a simple proteolytic precursor–product relationship for the two proteins. No immunoreactivity was discovered in immunoblots using peripheral organs; when crude solubilizates of rat and bovine liver, heart and kidney were compared with crude solubilizates of brain containing a similar amount of protein, only the brain samples were immunoreactive with a pattern similar to that of the purified receptor.

As bd-24 and bd-17 seemed to immunoreact with a $GABA_A$ receptor complex, we attempted to localize it immunohistochemically. An identical pattern of immunological staining was obtained with bd-17 and bd-24 in bovine or human brain sections, whereas in the rat only bd-17 was immunoreactive. (The lack of immunoreactivity of bd-24 in the rat was confirmed by immunoblotting and immunoprecipitation using a purified receptor preparation from rat brain.)

Assuming that the observed immunoreactivity in grey matter represented a $GABA_A$ receptor associated with a benzodiazepine receptor, we expected the staining pattern to be similar to that of benzodiazepine receptors visualized autoradiographically[16,17] by radioligand binding. Indeed, when semi-adjacent sections were incubated with the benzodiazepine receptor ligand ^{3}H-Ro 15-1788, the regional distribution and intensity of binding were strikingly similar to that of the immune reaction (Fig. 3), indicating that most benzodiazepine receptors are part of $GABA_A$ receptors. This was shown in rat brain, spinal cord and retina as well as in bovine and postmortem human brain (Fig. 3). Areas which lacked benzodiazepine receptor sites also lacked immunoreactivity, for example, brain white matter, pineal gland and pituitary.

In the granular layer of the rat cerebellum and the ventrolateral thalamus (Fig. 3*a*), the intensity of the immune reaction was higher than expected from the density of benzodiazepine binding sites (Fig. 3*b*), corresponding more accurately to the density of high-affinity GABA binding sites visualized autoradiographically with ^{3}H-muscimol (Fig. 3*c*). Thus, in these two regions the receptor complex may occur in a conformational

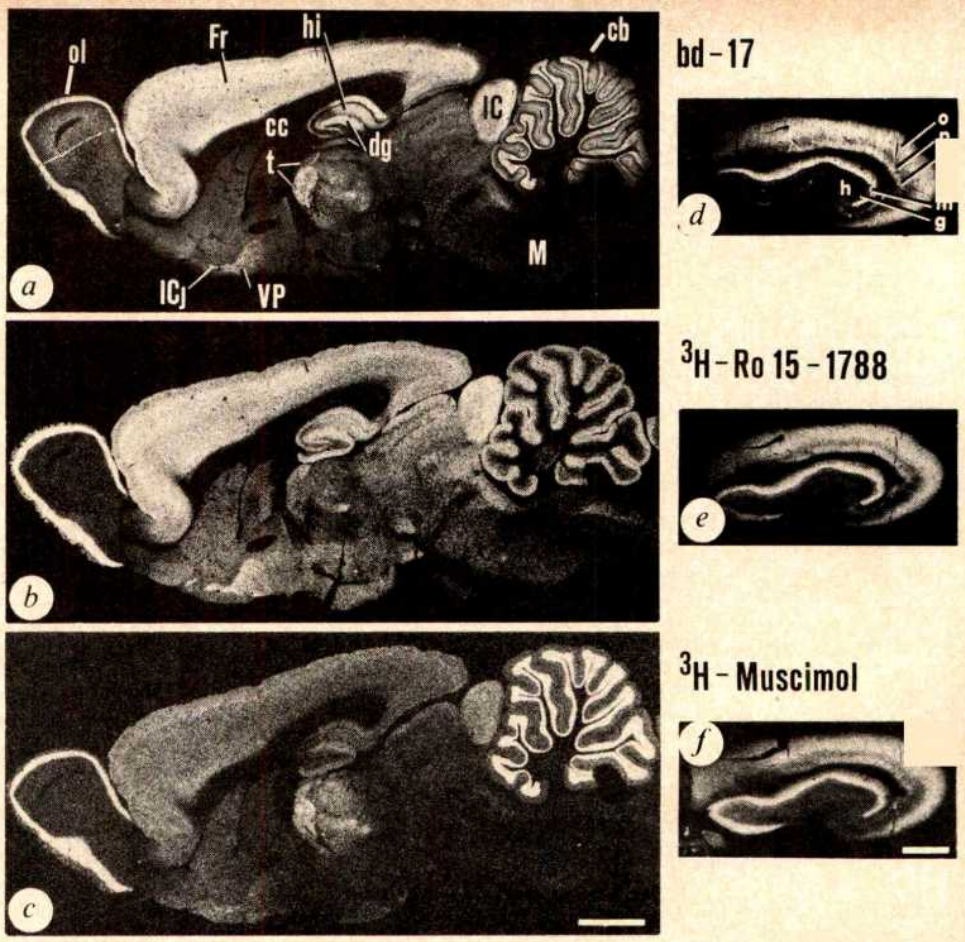


Fig. 3 Immunohistochemical localization of antigenic sites in rat brain (*a*, parasagittal section) and in postmortem human hippocampus (*d*, coronal section) using bd-17: comparison with the autoradiographic localization of benzodiazepine receptor sites (*b*, *e*; ^{3}H-Ro 15-1788 binding) and high-affinity GABA binding sites (*c*, *f*; ^{3}H-muscimol binding). In these negative images, white areas correspond to high densities of antigenic sites and radioligand binding sites.

Methods. *a*, *d*, For the immune staining the tissue was fixed with 2% formaldehyde + 0.1% glutaraldehyde in PBS. 10-μm cryostat sections were incubated, according to the indirect peroxidase/anti-peroxidase (PAP) technique[25], in sequence with: normal swine serum, hybridoma supernatant bd-17 (5–10 μg ml^{-1}), swine anti-mouse antiserum, PAP complex and finally diaminobenzidine/H_2O_2. In adsorption controls where bd-17 was preincubated with purified receptor complex, a nonspecific immunoreaction was observed, unless the receptor material was removed subsequently from the sample by a benzodiazepine receptor affinity resin[4]. Benzodiazepine receptor sites and high-affinity GABA binding sites were radiolabelled *in vitro* with 1 nM ^{3}H-Ro 15-1788 (*b*, *e*) and 5 nM ^{3}H-muscimol (*c*, *f*) respectively as described previously[16,20]. In the presence of 1 μM clonazepam and 10 μM GABA, binding of the respective radioligands was virtually absent. Abbreviations: cb, cerebellum; cc, corpus callosum; Fr, frontal cerebral cortex; dg, dentate gyrus; g, stratum granulosum of the dentate gyrus; h, hilus; hi, hippocampus; ICj, islands of Calleja; IC, inferior colliculus; M, medulla; m, stratum moleculare of the dentate gyrus; o, stratum oriens of the hippocampus; ol, olfactory bulb; p, stratum pyramidale of the hippocampus; r, stratum radiatum of the hippocampus; t, thalamus; VP, ventral pallidum. Scale bars, 2 mm.

state in which high-affinity GABA sites predominate and benzodiazepine binding sites are occluded. Alternatively, these areas may contain $GABA_A$ receptors not linked to benzodiazepine receptors but associated with Cl^- channels. The presumptive Cl^- channel ligand ^{35}S-*tert*-butylbicyclophosphorothionate[18], tested autoradiographically in rat cerebellum[19], showed the same pattern of distribution and density as the immune reaction in that brain region. If bd-17 and bd-24 cross-react with a $GABA_A$ receptor not linked to a benzodiazepine receptor, a structural homology between two types of $GABA_A$ receptors is suggested. The immunohistochemical staining does not seem to result from a nonspecific immune reaction, as preincubation of either antibody with the purified receptor fraction reduced the intensity of the immune reaction in a concentration-dependent fashion in all parts of the brain. Furthermore, controls with antibody-free medium or with an irrelevant antibody lacked immunoreactivity.

As benzodiazepines are used widely to treat anxiety states, sleep disorders, epileptic convulsions and muscle spasms, dysfunctions of the receptor complex may be involved in the aetiology of some of these disorders. Previous attempts to localize the receptor complex were restricted to the autoradiographical enhancement of radioligand binding to benzodiazepine receptors in micromolar concentrations of GABA[20], a technique of limited value for studies of postmortem human brain, as such receptor sites can be occupied through previous treatment with benzodiazepines. Furthermore, unknown amounts of endogenous GABA remaining in the tissue section could interfere with the enhancement of benzodiazepine receptor binding. In contrast, the immune reaction of bd-17 and bd-24 with the purified receptor remained unaltered in the presence of either GABA (5 mM) or diazepam (20 μM), as tested in a solid-phase antibody assay. Thus, an immunohistochemical investigation of postmortem tissue material can be performed irrespective of the presence of benzodiazepines or GABA and the structure containing the functionally relevant low-affinity GABA site can be visualized unequivocally with monoclonal antibodies. Furthermore, the resolution of the immunohistochemical localization of the receptor complex described here is superior to that of benzodiazepine receptor autoradiography, as single immu- noreactive cells with their processes can be visualized (J.G.R., in preparation). Thus, bd-17 and bd-24 may elucidate possible pathological alterations of the $GABA_A$ receptor/benzodiazepine receptor complex in postmortem human brain.

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1. Simmonds, M. A. in *Actions and Interactions of GABA and Benzodiazepines* (ed. Bowery, N. G.) 27–41 (Raven, New York, 1984).
2. Haefely, W. in *Actions and Interactions of GABA and Benzodiazepines* (ed. Bowery, N. G.) 263–285 (Raven, New York, 1984).
3. Haefely, W. & Polc, P. in *Anxiolytics: Neurochemical, Behavioral and Clinical Perspectives* (eds Malick, J. B., Enna, S. J. & Yamamura, H. I.) 113–146 (Raven, New York, 1983).
4. Schoch, P. & Möhler, H. *Eur. J. Pharmac.* **95**, 323–324 (1983).
5. Möhler, H. in *Receptor Chemistry* (eds Melchiorre, C. & Giannella, M.) 185–194 (Elsevier, Amsterdam, 1984).
6. MacDonald, R. & Barker, J. L. *Nature* **271**, 563–564 (1978).
7. Choi, D. W., Farb, D. H. & Fischbach, G. D. *J. Neurophysiol.* **45**, 621–631 (1981).
8. Alger, B. E. & Nicoll, R. A. *J. Physiol., Lond.* **328**, 125–141 (1982).
9. Smart, T. G., Constanti, A., Bilbe, G., Brown, D. A. & Barnard, E. A. *Neurosci. Lett.* **40**, 55–59 (1983).
10. Hamill, O. P., Bormann, J. & Sakmann, B. *Nature* **305**, 805–808 (1983).
11. Möhler, H., Battersby, M. K. & Richards, J. G. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1666–1670 (1980).

12. Sieghart, W. & Karobath, M. *Nature* **286**, 285-287 (1980).
13. Sigel, E., Stephenson, F. A., Mamalaki, C. & Barnard, E. A. *J. biol. Chem.* **258**, 6965-6971 (1983).
14. Sigel, E. & Barnard, E. A. *J. biol. Chem.* **259**, 7219-7223 (1984).
15. Schoch, P., Häring, P., Takacs, B., Stähli, C. & Möhler, H. *J. Recept. Res.* **4**, 189-200 (1984).
16. Richards, J. G. & Möhler, H. *Neuropharmacology* **23**, 233-242 (1984).
17. Young, W. S. & Kuhar, M. J. *J. Pharmac. exp. Ther.* **212**, 337-346 (1980).
18. Squires, R. F., Casida, J. E., Richardson, M. & Saedrup, E. *Molec. Pharmac.* **23**, 326-336 (1983).
19. Wamsley, J. K., Gee, K. W. & Yamamura, H. I. *Life Sci.* **33**, 2321-2329 (1983).
20. Unnerstall, J. R., Kuhar, M. J., Niehoff, D. L. & Palacios, J. M. *J. Pharmac. exp. Ther.* **218**, 797-804 (1981).
21. Gavish, M., Chang, R. S. L. & Snyder, S. H. *Life Sci.* **25**, 783-790 (1979).
22. Towbin, H., Staehelin, T. & Gordon, I. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350-4354 (1979).
23. De Blas, A. L. & Cherwinski, H. M. *Analyt. Biochem.* **133**, 214-219 (1983).
24. Takacs, B. J. & Staehelin, T. *Immun. Meth.* **2**, 27-56 (1981).
25. Sternberger, L. A. *Immunocytochemistry* (Wiley, New York, 1979).

Action potential mutations stop a biological clock in *Drosophila*

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The *Drosophila melanogaster* male produces a species-specific courtship song by wing vibration. The most conspicuous feature of the song is a series of pulses with a 30–40-ms interpulse interval (IPI)[1,2] which oscillate in wild-type males with a period of 50–60 s (ref. 3). This short-term biological rhythm in IPI is influenced by several gene mutations at the *period* (*per*) locus, which alter the normal 24-h free-running period of the circadian clock[4] and have corresponding effects on the song cycle[3]. The present study reveals that, under restrictive conditions, temperature-sensitive mutations which affect neuronal membrane excitability seem to stop the biological clock underlying the fruitfly's song rhythm.

We have taken advantage of two temperature-sensitive mutations: nap^{ts} (no-action-potential temperature-sensitive) and $para^{ts1}$ (paralytic temperature-sensitive). Flies carrying these mutations are immobilized rapidly at temperatures >35 °C and 29 °C, respectively[5,6], but this paralysis is reversible, both mutants recovering within seconds when returned to 25 °C. At the higher restrictive temperatures, both mutants fail to generate action potentials[7]. These defects in nerve conduction may be caused by abnormalities associated with regenerative sodium channels. Our experimental design allowed individual nap^{ts} and $para^{ts1}$ males to begin courtship singing to normal females at 25 °C, then heat pulses were applied for 30 s to 'switch off' the male's nervous system. On recovery at 25 °C each male was permitted to continue his courtship. Pre- and post-treatment songs were then analysed to determine whether any phase shifts in the song cycle had occurred.

To control for the effects of the 30-s paralysis induced by failure of nerve conduction in the mutants, normal and mutant males were incapacitated for the same period of 30 s with short 7-s pulses of low temperature or CO_2 (see Table 1 and Fig. 1 legend). These control tests almost certainly do not obliterate membrane excitability except perhaps for a brief period within the 7-s pulse (see, for example, refs 8–11). In the analogous

Table 1 Induced phase shifts of the courtship song rhythm in *Drosophila* males

Genotype	Treatment	*n*	Mean phase shift (s) ± s.e.m. (advances = +, delays = −)	Range (s)
Experimental				
Wild type	30-s heat pulse (42 °C)	5	+1.5 ± 1.8	−4.9 to +5.0
nap^{ts}	30-s heat pulse (42 °C)	7	−31.2 ± 1.8	−38.2 to −26.1
$nap^{ts}cn$	30-s heat pulse (37 °C)	8	−28.9 ± 2.5	−39.1 to −21.3
$para^{ts1}$	30-s heat pulse (37 °C)	8	−31.0 ± 3.1	−39.0 to −16.1
$nap^{ts}cn$	15-s heat pulse (37 °C)	6	−16.2 ± 3.2	−25.2 to −2.0
$para^{ts1}$	15-s heat pulse (37 °C)	7	−13.0 ± 2.8	−23.8 to −3.9
$nap^{ts}cn$	45-s heat pulse (37 °C)	5	−48.4 ± 4.6	−59.3 to −36.3
$para^{ts1}$	45-s heat pulse (37 °C)	4	−47.5 ± 2.8	−53.9 to −40.8
Controls				
Wild type	Removed from female only	6	+1.0 ± 2.6	−6.7 to +9.9
$nap^{ts}cn$	Removed from female only	6	+0.5 ± 2.5	−9.9 to +7.0
$para^{ts1}$	Removed from female only	5	+1.3 ± 3.2	−8.0 to +8.0
Wild type	7-s cold pulse (0 °C)	4	+0.2 ± 2.4	−4.0 to +5.0
nap^{ts}	7-s cold pulse (0 °C)	4	−4.0 ± 1.3	−7.0 to −0.7
Wild type	7-s CO_2 pulse	5	−2.3 ± 1.2	−4.7 to +5.7
nap^{ts}	7-s CO_2 pulse	5	−1.8 ± 2.2	−7.1 to +5.3

Two strains carrying the chromosome 2 mutation nap^{ts} were used: one of these had been maintained at the California Institute of Technology at 25 °C and these flies were quickly immobilized at 42 °C. A second line, carrying the eye colour mutation *cinnabar* (*cn*) had been maintained at 18 °C by Dr Barry Ganetzky at the University of Wisconsin. The sex-linked $para^{ts1}$ mutation has also been maintained at 18 °C in Wisconsin, and both $nap^{ts}cn$ and $para^{ts1}$ flies showed nearly instantaneous paralysis at 37 °C. The males were placed in a preheated glass tube at either 37 °C or 42 °C and maintained at this temperature for 15, 30 or 45 s, as indicated. nap^{ts} and $para^{ts1}$ males became immobilized within 1–2 s at these temperatures and remained motionless for the duration of the heat pulse. The mutants were then removed from the tubes and replaced in the mating chamber at 25 °C where they regained full mobility within 2–5 s. The wild-type males did not 'pass out' during similar high-temperature treatment. Females were then re-introduced to the males. In the no-treatment controls, the male was simply removed from the female for 60 s and then returned. In this test the total time for which the male was removed from the female (including 20–25-s handling time) was 80–85 s, which corresponds to approximately $1\frac{1}{2}$ cycles of the normal 55-s song rhythm. Thus, if this treatment arrested the clock, the song rhythm would be approximately half a cycle out of phase when the male resumed his courtship. In a further control test, the male was immobilized in a chilled glass tube at 0 °C. All flies were immediately disabled at this temperature and usually remained in torpor for 25–35 s if the cold pulse was maintained for no longer than 7 s. Any male remaining motionless for less than 25 or more than 35 s was discarded. In a final control treatment, the courting male was removed from the female and placed in a small mating cell, into which CO_2 was blown for 7 s. Only those males that were anaesthetized and remained motionless for between 25 and 35 s were subsequently returned to the female. After each treatment males were allowed to continue their courtship for 5–8 min. Flies that had not resumed courtship 5 min after the end of the pulse were eliminated from further analysis. Males which did not sing vigorously either before or after the treatment were similarly discarded. Between 10 and 15 males were observed initially in each experiment but many did not court at all, either before or after the treatment, or sang so weakly that further analysis was not warranted. A vigorous courtship was operationally defined as one in which an average of 25 individual IPIs could be measured per 10 s of courtship time. Phase shifts were computed as described in Fig. 1 legend. Of the 170 songs analysed above (85 pre- and 85 post-treatment songs), 113 were significantly rhythmic beyond the 1% level (see ref. 3), 51 were significantly rhythmic beyond the 5% level and 6 just missed significance at 5%. In addition, 14 males sang vigorously but were arrhythmic in either their pre- or post-treatment songs. *n*, The number of males in each experimental group whose courtship song was analysed. Males were tested once only.

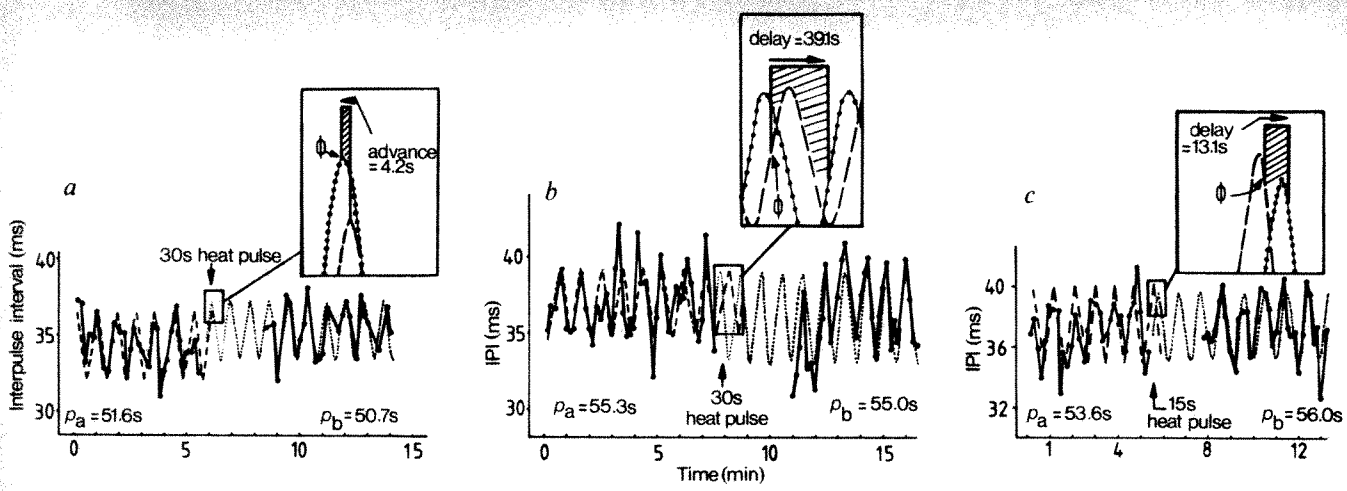


Fig. 1 Induced phase shifts of the song rhythms of *nap*^{ts}*cn* and wild-type *Drosophila* males. Each point represents the mean IPI per 10 s of courtship time (values of s.e.m. have been omitted for clarity, but 80% of all the s.e.m. values fall in the range 0.4–1.2 ms). Tape recordings of the courtship songs of 5-day-old males to 2-day-old virgin, attached-X chromosome females were made from a small mating cell using a ribbon microphone and were then input to an oscillograph to provide a visual record of the pulse trains[3]. IPIs were measured peak-to-peak with a ruler using minimum and maximum cut-off points of 15 and 65 ms, respectively. Song rhythms were obtained by calculating the mean IPI for successive 10-s time frames and placing these means into a computer together with approximate initial values for the parameters of a sine function. These parameters included the phase, period and amplitude of the rhythm, and the value around which the cycle oscillated. An iterative programme was run twice for each male's song, once to obtain the best-fitting sine wave for the pretreatment IPI means (dashed line) and then again for the post-treatment means (dotted line). The computer analysis was performed only after both sets of data (that is, pre- and post-treatment IPI means) had been calculated for each male. The goodness-of-fit of each set of data to its corresponding sine wave was examined using an *F*-test, yielding a measure of how 'sinusoidal' or 'rhythmic' the data were. Estimates of the various sine-wave parameters were used to calculate the phase shift between the pre- and post-treatment song rhythms. Because of the frequent small differences in the periods of the pre- and post-treatment songs (p_a and p_b, respectively), the phase shift between the two curves was calculated at the moment the treatment pulse was applied. ***a***, Song profile for a wild-type male subjected to a heat pulse (42 °C) for 30 s. The pretreatment song period, p_a, is 51.6 s: $p_b = 50.7$ s. The heat pulse was administered 370 s after the beginning of the courtship song, and when the pre-treatment song rhythm is extrapolated to this point, the phase at which the heat stimulus was applied, Φ, is 1.57 radians (this value is the peak of the cycle). Extrapolating the post-treatment song rhythm back to this point, the time coordinate which represents a phase of 1.57 radians for the post-treatment curve is 365.8 s. Thus the two peaks are 370 − 365.8 s, or +4.2 s, out of phase. This represents an advance of 4.2 s of the post-treatment song rhythm on that of the pretreatment. The shaded area in the inset represents this phase advance. ***b***, The song rhythm of a *nap*^{ts}*cn* male undergoing a paralysing 30-s heat pulse (37 °C) administered 470 s after the male initiated courtship singing. This time point corresponds to a phase of 5.95 radians on the pretreatment curve. The corresponding coordinates for Φ = 5.95 radians on the post-treatment curve fall at 454.1 s and then at 509.1 s on the following cycle, yielding phase shifts of either 470 − 454.1 = +15.9 s (advance), or 470 − 509.1 = −39.1 s (delay). We assumed initially that this phase shift was a delay (see text). The shaded area in the inset again represents the phase delay. ***c***, The song produced by a *nap*^{ts}*cn* male subjected to a 15-s paralysing heat pulse (37 °C), at 340 s. This time point represents Φ = 1.92 radians (again like ***a***, the heat pulse is initiated near the peak of the cycle) on the pretreatment curve. The corresponding time coordinate represented by this phase angle falls at 353.1 s on the post-treatment curve. The phase shift is therefore 340 − 353.1 = −13.1 s (delay).

mutant cases, action potentials are blocked for the duration of the paralysis. The no-treatment control represents a systematic test of our earlier observation that when a male resumes singing after previously interrupting courtship, the song cycles remain in-phase with those established earlier[3].

Table 1 summarizes the phase shifts induced by the various treatments and Fig. 1 illustrates some individual results. Mutant and wild-type males which received no treatment, except for a 60-s absence from the female, exhibited mean phase shifts of <2 s. Wild-type males subjected to 42° C pulses also showed small phase shifts. In the case of the mutants undergoing 30-s high-temperature pulses, we assumed initially that all phase shifts were delays; in these three groups the song rhythms were phase-delayed by ~30 s. In contrast, mutant and wild-type flies immobilized for between 25 and 35 s with 'cold' or CO_2 pulses showed small (<5-s) phase shifts. The small phase shifts in the controls can be classified clearly as advances or delays. The large (~30 s) phase shifts in the heat-pulsed mutants, however, are more difficult to categorize because 'delays' greater than half the song period can also be defined as advances. In Fig. 1*b*, which illustrates the song profile of a *nap*^{ts}*cn* male subjected to a 30-s heat pulse, the large 39.1-s delay could also be interpreted as an advance of 15.9 s. We therefore applied 15-s heat pulses to courting *nap*^{ts}*cn* and *para*^{ts1} males in the same conditions as before; we observed that all phase shifts could be unambiguously categorized as delays (that is, <180°), with the average phase shifts for the two mutant groups being ~15 s (see Table 1). Thus, we believe that the previous 30-s heat treatments similarly caused delays in the mutants. To confirm this interpretation of the data, we also applied 45-s heat pulses to courting *nap*^{ts}*cn* and *para*^{ts1} males. Our results supported the delay hypothesis in that large delays of ~45 s were observed in both groups (see Table 1). Thus, we conclude that 'shutting down' the nervous system in these mutants with heat pulses leads to a stoppage of the song clock proportional to the duration of the heat stimulus.

This is a novel result which does not have a parallel in the circadian literature. Circadian rhythms often reveal phase response characteristics when pulses of heat or cold are applied to a 24-h rhythm at various points in its cycle[12–14]. In the *nap*^{ts} and *para*^{ts} mutants, however, the song clock simply seems to 'stop' at restrictive temperatures and to start again when the fly is returned to the permissive temperature. Unfortunately, because of the small numbers of flies tested, it was difficult to determine whether there were any phase-response patterns in the songs of wild-type flies undergoing temperature pulses. Larger scale experiments using wider ranges of hot and cold pulses may be required if any phase-response curves are to be obtained for the song rhythm.

The *nap*^{ts} and *para*^{ts} mutations which we have used to block neuronal conductance act synergistically in that double mutants do not survive at any temperature[7], suggesting that the mutations share a common membrane excitability function. *nap*^{ts} seems to alter the density of sodium channels as deduced by tetrodotoxin (TTX) and saxitoxin binding studies[15–17], and wild-type flies given a sub-lethal dose of TTX, which blocks and therefore reduces the number of membrane-bound sodium channels, show temperature-sensitive paralysis similar to that of *nap*^{ts} flies[15]. Perhaps, therefore, the two mutations interrupt the

Drosophila song clock because of the direct[7] or indirect[15] effects of these genetic variants on the structure and function of sodium channels in neural membranes. Pharmacological manipulations of ionic fluxes change the phasing of a circadian oscillator localized in the *Aplysia* eye[18,19] but TTX does not phase-shift this neural rhythm[20]. In view of the latter result, it would be useful to examine whether the nap^{ts} and $para^{ts}$ mutations lead to a temperature-sensitive arrest of the circadian clock in flies. There is no reason to assume that every perturbation which affects the song clock will similarly affect the circadian clock, even though both rhythms share a common genetic mechanism via the effects of the *per* mutations[3]. For example, constant light disrupts the circadian clock, but not the song oscillation[3]. Therefore, the nap^{ts} and $para^{ts}$ mutations can be used to probe the differences as well as the similarities between short-cycling and circadian rhythms. The striking stoppage of the fly's song clock by block of neuronal conductance with the nap^{ts} or the $para^{ts}$ mutations indicates that this short-term rhythm is regulated by a neural oscillator. Genetic procedures[21] are available for localizing these presumptive neural 'foci', by constructing genetic mosaics which express nap^{ts} or $para^{ts}$ in their mutant tissues. Such studies may reveal whether these mutations lead to an interruption of the song cycle by affecting a small localized region within a ganglion or whether several sites (each perhaps containing a separate oscillator) must be simultaneously mutant in order for such arrests to be observed. In the latter case, communication may be blocked between oscillators which need to be coupled to regulate the phase of the rhythm; these foci may be the same or quite different from the sites of action of the *per* gene. Mosaic experiments on circadian behaviour have shown that the anatomical focus for the short-period per^s variant is localized in the fly's head[22]. Further work on the effects of the nap^{ts}, $para^{ts}$ and *per* mutations should help to elucidate the complex relationship between the mechanisms controlling short-term and circadian rhythms in *Drosophila*.

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1. Ewing, A. W. & Bennett-Clark, H. C. *Behaviour* **31**, 287-301 (1968).
2. von Schilcher, F. *Anim. Behav.* **24**, 18-26 (1976).
3. Kyriacou, C. P. & Hall, J. C. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6729-6733 (1980).
4. Konopka, R. & Benzer, S. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2112-2116 (1971).
5. Wu, C. F., Ganetsky, B., Jan, L. Y., Jan, Y. N. & Benzer, S. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4047-4051 (1978).
6. Suzuki, D. T., Grigliatti, T. & Williamson, R. *Proc. natn. Acad. Sci. U.S.A.* **68**, 890-893 (1971).
7. Wu, C. F. & Ganetzky, B. *Nature* **28**, 814-816 (1980).
8. Kimura, J. E. & Meves, H. *J. Physiol., Lond.* **289**, 479-500 (1979).
9. Sevcik, C. *J. Physiol., Lond.* **352**, 187-194 (1982).
10. Fuzeau-Braesch, S. & Nicholas, N. *Comp. Biochem. Physiol.* **68A**, 289-297 (1981).
11. Clark, M. A. & Eaton, D. C. *J. Neurobiol.* **14**, 237-250 (1983).
12. Pittendrigh, C. S. *Proc. natn. Acad. Sci. U.S.A.* **40**, 1018-1029 (1954).
13. Saunders, D. S. *Insect Clocks* 2nd edn (Pergamon, Oxford, 1982).
14. Takahashi, J. S. & Zatz, M. *Science* **217**, 1104-1111 (1982).
15. Jackson, F. R., Wilson, S. D., Strichartz, G. R. & Hall, L. M. *Nature* **308**, 189-191 (1984).
16. Kauvar, L. M. *Molec. gen. Genet.* **187**, 172-173 (1982).
17. Hall, L. M., Wilson, S. D., Gischier, J., Martinez, N. & Strichartz, G. R. *Ciba Fdn Symp.* **88**, 207-220 (1982).
18. Eskin, A. & Correnti, G. *J. comp. Physiol.* **117**, 1-21 (1977).
19. Eskin, A. *J. Neurobiol.* **13**, 241-249 (1981).
20. Eskin, A. *J. Neurobiol.* **8**, 273-299 (1977).
21. Hall, J. C. in *Results and Problems in Cell Differentiation* Vol. 9 (ed. Gehring, W. J.) (Springer, Berlin, 1978).
22. Konopka, R., Wells, S. & Lee, T. *Molec. gen. Genet.* **190**, 284-288 (1983).

Comparative chromosome mapping of a conserved homoeo box region in mouse and human

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Specific genes are assumed to regulate pattern formation in the mammalian embryo, but as yet none has been identified unequivocally. It is possible that such genes in mammals may be identified by virtue of a conserved coding sequence, because many of the *Drosophila melanogaster* homoeotic and segmentation genes, which have crucial roles in the regulation of segmental pattern formation during embryonic development[1,2], contain a 180-base pair (bp) DNA sequence, the homoeo box[3-5], and that sequences homologous to the *Drosophila* homoeo box are also present in 6–10 copies in higher animals, including mammals[4-8]. Although the assumption that the homoeo box identifies genes responsible for pattern formation in mammals remains to be validated, it is a particularly attractive hypothesis given the strong conservation of homoeo boxes over vast evolutionary distances. Here we report the localization of a human homoeo box region[7], previously cloned and shown to contain two homoeo boxes within a sequence of 5-kilobases (kb), to the long arm of chromosome 17. We show that two single-copy homoeo box-flanking probes derived from this region strongly hybridize to single-copy restriction fragments in mouse genomic DNA and that these conserved homoeo box-flanking sequences map to mouse chromosome 11. This may be significant as several genes that map to chromosome 17 in human also map to chromosome 11 in the mouse, implying that a segment of mouse chromosome 11 is homologous to a region of human chromosome 17 (ref. 9). Taken together, these data suggest that the homoeo box region detected with our probes is highly conserved in human and mouse.

To determine the chromosomal location of the human homoeo box region, two single-copy probes were constructed. Figure 1 shows a restriction map of the cloned region of the human genome, containing two linked homoeo boxes[7] designated *Hu-1* and *Hu-2*, and illustrates the probes derived from it. One probe, H.1, is a 1.7 kb *Hin*dIII fragment containing the *Hu-1* homoeo box; the other probe, LF.2, is a 1.2-kb *Hin*dIII/*Eco*RI fragment containing sequences flanking the *Hu-2* homoeo box on the side opposite *Hu-1* or on the left side of *Hu-2* as the map is presented. Southern blot analysis has shown that the H.1 probe detects a single restriction enzyme fragment in human DNA when

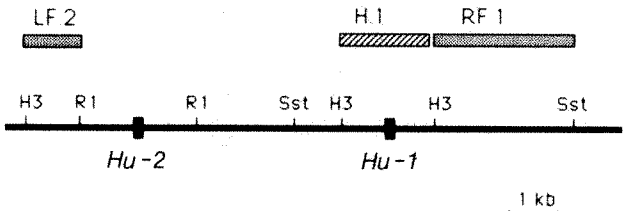


Fig. 1 Restriction map of the human homoeo box region studied. The probes used for the mapping studies are indicated. The restriction map was derived from two clones, λHu1 and λHu2, described elsewhere[7]. The horizontal line represents the human DNA sequence and the two small solid boxes represent homoeo boxes. The exact position of each of the homoeo boxes between the two restriction sites indicated has not been determined. The stippled and cross-hatched boxes above the restriction map represent the probes derived from this region. LF.2, or left flanking sequence to *Hu-2*, is a 1.2-kb *Eco*RI/*Hin*dIII fragment; H.1 is a 1.7-kb *Hin*dIII fragment containing the *Hu-1* homoeo box; and RF.1, or right flanking sequence to *Hu-1*, is a 2.4-kb *Hin*dIII-*Sst*I fragment. These fragments were subcloned into the vector SP65 and the recombinant plasmids (pLF.2, pH.1 and pRF.1) were used as probes. R1, *Eco*RI; H3, *Hin*dIII; Sst, *Sst*I.

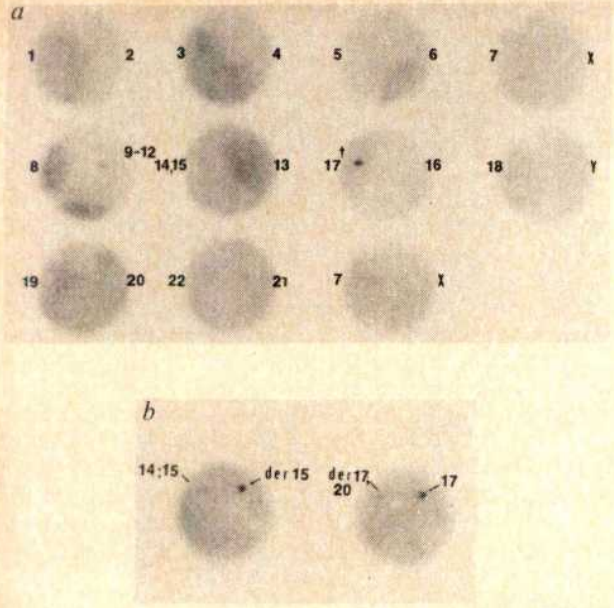


Fig. 2 Assignment of the human homoeo box region to the long arm of chromosome 17. *a*, Autoradiograph of a panel of spot blot filters containing flow-sorted human chromosomes hybridized to the LF.2 probe. *b*, Autoradiograph of spot blot filters containing flow-sorted chromosomes from a cell line containing a 15;17 reciprocal translocation hybridized to the LF.2 probe.
Methods. Chromosomes (30,000) of each type were either sorted directly onto spots on nitrocellulose filters, denatured and neutralized as previously described[10,11] or sorted directly into Leif buckets and identified by quinicrine-banding analysis. The filters were prehybridized overnight at 42 °C in 5×SSC, 5×Denhardt's solution, 250 μg ml^{-1} sonicated boiled salmon sperm DNA, 50 mM $NaPO_4$, *p*H 7, 0.1% SDS and 50% deionized formamide and hybridized for 48 h at 42 °C in the same solution containing nick-translated ^{32}P-labelled pLF.2 (3×10^6 c.p.m. ml^{-1}). After hybridization, the filters were washed three times for 5 min each at room temperature and twice at 50 °C for 30 min each in 2×SSC and 0.1% SDS, followed by one wash in 0.2×SSC for 30 min at 65 °C.

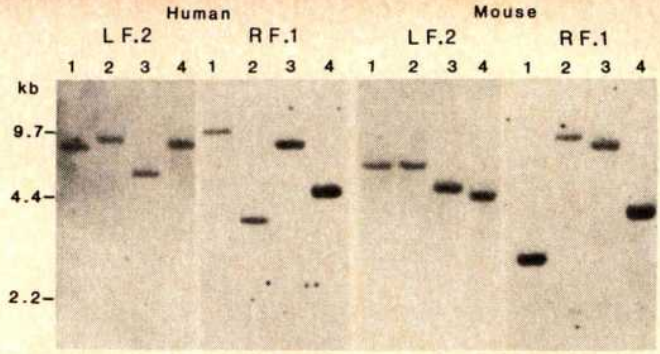


Fig. 3 Hybridization of the homoeo box flanking probes, LF.2 and RF.1, to human and mouse DNAs. The DNAs were restricted with the following enzymes: 1, *Eco*RI; 2, *Bam*HI; 3, *Hin*dIII; 4, *Sst*I.
Methods. Total cellular DNAs (10 μg) isolated from human and mouse tissues were cleaved with the restriction enzymes indicated. After gel electrophoresis and transfer to nitrocellulose, the baked filters were hybridized to pLF.2 and pRF.1 probes as described in Fig. 2 legend, except that the last wash in 0.2×SSC was at 50 °C and in some cases the filters were prehybridized and hybridized at 64 °C in 6.6×SSC, 10×Denhardt's, 0.1% SDS, 0.1% sodium pyrophosphate.

hybridized and washed in stringent conditions[7]. LF.2 also hybridizes to a single-copy sequence in human genomic DNA (see below).

To determine which human chromosome contains the homoeo box region, we used dual-laser chromosome sorting and spot-blot analyses[10,11]. Two panels of human chromosomes sorted directly onto nitrocellulose filters (spot blots) were hybridized with the H.1 and LF.2 probes and washed at high stringency (see Fig. 2 legend). Figure 2*a* shows an autoradiograph of a spot blot hybridized to LF.2. A specific signal is detected only in the fraction containing chromosome 17; the weak signal detected in the fraction containing a pool of chromosomes 9–12 is observed with most probes and is presumably nonspecific background which is more prominent in this dot owing to the presence of more DNA than in dots containing only one chromosome. As expected, hybridization of a similar panel of chromosomes to H.1 gives an identical result (data not shown).

In order to sub-localize the homoeo box-related sequences on chromosome 17, we sorted chromosomes from human fibroblasts heterozygous for a reciprocal translocation between the long arms of chromosomes 17 and 15 [46 XX, t(15; 17)(q22; q11)] (M. LeBeau and J. Rowley, personal communication). The normal chromosome 17 and the derivative 15 [der(15)], which contains the long arm of chromosome 17, are each sorted separately, whereas the derivative 17 [der(17)] which contains the short arm of chromosome 17, is sorted with chromosome 20 (not shown). Chromosomes 14 and 15 are sorted together and were spot-blotted as a negative control. Figure 2*b* shows an autoradiograph of the spot blot from this chromosome sort hybridized to the LF.2 probe. A specific signal is detected in the two fractions containing chromosome 17 and [der(15)]. Thus, these experiments localize the human homoeo box region shown in Fig. 1 to the long arm of chromosome 17.

Comparative gene mapping has demonstrated genetic homology between the long arm of human chromosome 17 and mouse chromosome 11, hence if the homoeo box region on human chromosome 17 is conserved in the mouse, it may map to mouse chromosome 11 (ref. 9). To test this hypothesis, we first demonstrated that DNA sequences homologous to the human chromosome 17 homoeo box region are present in mouse genomic DNA. In addition to the *Hu-2*-flanking probe LF.2, we constructed a 2.4-kb *Hu-1*-flanking probe (RF.1) containing sequences adjacent to *Hu-1* on the side opposite *Hu-2* or on the right side of *Hu-1* (see Fig. 1). The Southern blot experiments shown in Fig. 3 demonstrate that the LF.2 and RF.1 probes detect specific restriction fragments in human DNA cut with a variety of enzymes, indicating that each probe recognizes a different single-copy sequence in human DNA. The fragment sizes detected are those predicted from the restriction map of the human clones shown in Fig. 1. To demonstrate homology between these flanking probes and mouse DNA sequences, we hybridized a Southern blot of mouse genomic DNA restricted with a variety of enzymes to LF.2, and RF.1 under moderate stringency (see Fig. 3 legend). Figure 3 shows the results of such an experiment. The *Hu-1*- and *Hu-2*-flanking probes detect specific restriction enzyme fragments with each of the enzymes used.

To determine whether the single-copy mouse DNA sequences homologous to the probes LF.2 and RF.1 map to mouse chromosome 11, we used rat-mouse and Chinese hamster-mouse somatic cell hybrids. RTM9, a rat-mouse somatic cell hybrid clone, was formed by polyethylene glycol-mediated fusion of thymidine kinase-deficient Rat-2 cells with spleen cells from a mouse homozygous for an 11;13 robertsonian translocation chromosome, followed by selection in hypoxanthine-aminopterin-thymidine medium[12]. The murine 11;13 translocation chromosome is the only mouse chromosome in this hybrid clone and is present in over 90% of RTM9 metaphases analysed by standard trypsin-Giemsa banding techniques (data not shown). The mouse thymidine kinase gene is present on mouse chromosome 11. Thus, by growing RTM9 in medium containing 5-bromodeoxyuridine, we were able to isolate a subclone, RTM9-Bu, that lacks the mouse 11;13 chromosome but retains the same rat chromosome complement as the parental clone, as determined by karyotype analysis. We also tested a Chinese hamster-

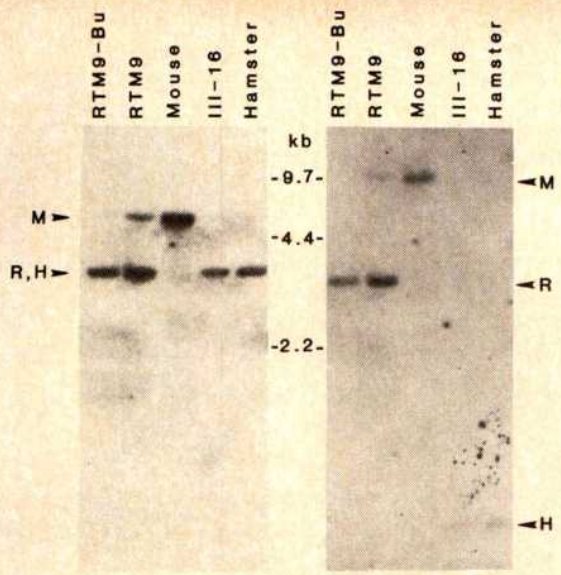


Fig. 4 Detection of mouse-specific LF.2 and RF.1 homologous sequences in DNA from the hybrid cell line RTM9, which contains mouse chromosomes 11 and 13, and demonstration of their absence from the cell hybrid III-16, which contains several mouse chromosomes including 13, but not 11. R, rat; M, mouse; H, hamster. **Methods.** Total cellular DNAs were isolated from normal mouse tissues, the rat–mouse somatic cell hybrid RTM9, the back-selected subclone RTM9-Bu, which contains no mouse chromosomes but retains the same complement of chromosomes as the parental clone, the Chinese hamster–mouse hybrid III-16, and the hamster cell line 380-6 that was used to construct III-16. These DNAs were cleaved with *Hin*dIII, Southern-blotted and hybridized to pLF.2 and pRF.1 probes as described in Fig. 3 legend.

mouse somatic cell hybrid, III-16 (ref. 12), containing mouse chromosome 13 as well as several other mouse chromosomes but lacking mouse chromosome 11. Genomic DNA was isolated from normal mouse tissues, RTM9, RTM9-Bu, III-16, and the Chinese hamster parental cell line 380-6 that was used to construct III-16. The murine DNA fragments detected by LF.2 and RF.1 were distinguished from the homologous hamster or rat DNA fragments on the basis of size after *Hin*dIII restriction enzyme digestion, followed by Southern transfer and hybridization at moderate stringency (see Fig. 3 legend). Figure 4 shows that the mouse-specific DNA fragments that hybridize with the LF.2 and RF.1 probes are present in RTM9 DNA, but not in DNA from RTM9-Bu or III-16. Results of a similar study using the H.1 probe were difficult to interpret because, in the moderate hybridization conditions used, this homoeo box-containing probe detects 6–8 DNA fragments in mouse, hamster and rat DNA. Taken together, these results demonstrate that the single-copy DNA sequences homologous to those in the human chromosome 17 homoeo box region are linked in the mouse, as they are in human, and map to chromosome 11.

The extent to which the homoeo box region is conserved between human and mouse will remain unknown until the homologous mouse *Hu-1*- and *Hu-2*-flanking sequences have been cloned and sequenced. The availability of the murine genomic clones should also make it possible to determine the number of homoeo boxes in this region. By analogy with human, we expect that this region of mouse chromosome 11 contains at least two homoeo boxes. This suggests that there may be clustering of homoeo boxes in mammals, as is the case in *Drosophila*. However, not all homoeo box-containing sequences map to mouse chromosome 11—at least one homoeo box sequence maps to mouse chromosome 6 (ref. 8), indicating that there is some dispersion of homoeo boxes in the mouse genome.

The fact that the human chromosome 17 homoeo box region is conserved in mouse suggests that this region is indeed part of a coding sequence in the mammalian genome. This conclusion is based on data from comparative sequence studies of gene families such as the globin genes, which demonstrate that coding regions are strongly conserved whereas introns and flanking sequences are generally not conserved[13]. Whether the genes found within this human/mouse homoeo box region have an important role in the control of mammalian development remains to be determined. In this context it may be relevant that two mutations that affect the number and form of the vertebrae have been mapped[14] to mouse chromosome 11: *vestigial-tail* is a viable recessive mutation, and *tail-short* is a dominant mutation which is viable in the heterozygous condition, but which causes embryonic death in the peri-implantation period when homozygous. It will be of interest to determine whether either of these mutations is allelic to the mouse cognate of the human homoeo box region that we have assigned to mouse chromosome 11.

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1. Ouweneel, W. J. *Adv. Genet.* **18**, 179–248 (1976).
2. Nusslein-Volhard, C. & Wieschaus, E. *Nature* **287**, 795–801 (1980).
3. McGinnis, W., Levine, M., Hafen, E., Kuroiwa, A. & Gehring, W. J. *Nature* **308**, 428–433 (1984).
4. McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. & Gehring, W. J. *Cell* **37**, 403–408 (1984).
5. Scott, M. P. & Weiner, A. J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4115–4119 (1984).
6. Carrasco, A. E., McGinnis, W., Gehring, W. J. & De Robertis, E. D. *Cell* **37**, 409–414 (1984).
7. Levine, M., Rubin, G. M. & Tjian, R. *Cell* **38**, 667–673 (1984).
8. McGinnis, W., Hart, C. P., Gehring, W. J. & Ruddle, F. H. *Cell* **38**, 675–680 (1984).
9. Roderick, T. H. *et al. Cytogenet. Cell Genet.* **37**, 321–339 (1984).
10. Lebo, R. V. *et al. Science* **225**, 57–59 (1984).
11. Lebo, R. V. *et al. Hum. Genet.* (in the press).
12. Cox, D. R., Sawicki, J. A., Yee, D., Apella, E. & Epstein, C. J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1930–1934 (1982).
13. Nishioka, Y. & Leder, P. *Cell* **18**, 875–882 (1979).
14. Green, M. C. (ed.) *Genetic Variants and Strains of the Laboratory Mouse* (Fischer, New York, 1981).

Two homoeo box loci mapped in evolutionarily related mouse and human chromosomes

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The homoeo box is a 180-base pair (bp) DNA sequence conserved in *Drosophila* homoeotic genes, which regulate early development[1,2]. These DNA sequences are present in open reading frames and have been identified in specific gene transcripts in *Drosophila* and *Xenopus* embryos[3–5]; they possess structural features in common with genes encoding some DNA-binding proteins[6,7]. Homologous homoeo box sequences have been detected in species ranging from insects and annelids to vertebrates[2,5,8]. The high degree of sequence conservation (70–90%) among different species suggests a strong evolutionary relationship and implies a common role in embryonic development. To test this hypothesis, one approach we have used is to examine the patterns of genetic organization of homoeo box sequences in mouse and human for any similarities; the second approach is to localize the chromosomal map positions of homoeo box sequences in the two species. A similar genomic organization and chromosomal distribution of homoeo box sequences would argue for a conserved function and might shed light on their mechanism of action. Here, we describe experiments which show that two homoeo box loci map, respectively, to evolutionarily related regions on mouse chromosome 11 and human chromosome 17.

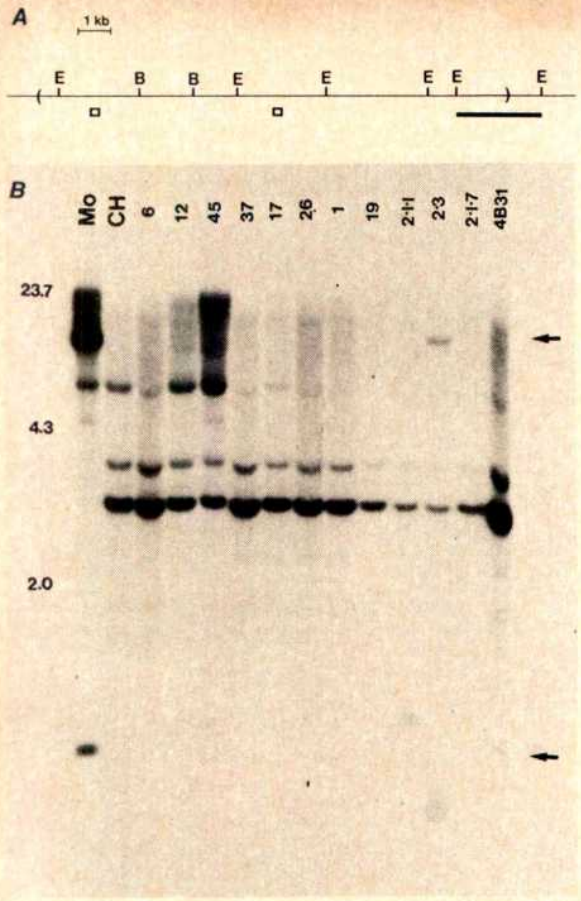


Fig. 1 *A*, Restriction map of the *Hox-2* recombinant phage insert. Boxes beneath the line indicate the two restriction fragments containing sequences homologous to the *Drosophila* homoeo box. The hybridization probe used for chromosomal mapping is underlined by the solid bar. The brackets indicate vector/insert cloning sites. B, *Bam*HI; E, *Eco*RI. *B*, Southern blot hybridization of mouse, hamster and mouse×hamster hybrid cell DNAs with the *Hox-2* DNA probe indicated in *A*. The *Hox-2* probe was nick-translated with ^{32}P-dCTP to a specific activity of 10^8 c.p.m. μg^{-1} and hybridized as reported previously[9]. The murine chromosome complement of the hybrid cell lines was determined by karyotyping and/or analysis of isozyme and DNA markers.

A recombinant phage clone containing murine DNA homologous to *Drosophila* homoeo box sequences was isolated from a mouse genomic library as reported previously[9]. Restriction site mapping of the murine DNA insert described here showed that this clone was not derived from the homoeo box locus (*Hox-1*) present on mouse chromosome 6 (ref. 9), hence we call this new region *Hox-2*. Southern blot hybridization of restriction enzyme-digested *Hox-2* DNA with a *Drosophila* homoeo box probe revealed two distinct regions of homoeo box sequence homology, 5 kilobases (kb) apart. This structural organization was similar to that reported for two linked homoeo boxes in the human genome (*Hu-1* and *Hu-2*)[8], and suggests that the *Hox-2* locus in the mouse and the region spanning the *Hu-1* and *Hu-2* probes in man may represent homologous genomic domains. As an initial step in the comparison of these homoeo box regions in mouse and human, we have mapped these loci to determine whether they are localized in evolutionarily related chromosomal regions.

To map the cloned mouse sequences, 20 mouse×hamster hybrid cell DNAs were probed with a 2.7-kb *Eco*RI fragment flanking the homoeo box regions of the *Hox-2* recombinant phage insert (Fig. 1*A*). The murine DNA contained in this fragment hybridizes with 0.9-kb and 10-kb *Hin*dIII fragments in a mouse genomic Southern blot. A strongly cross-hybridizing band of 3 kb is present in the hamster genome. In addition,

Table 1 Segregation of *Hox-2* in mouse-hamster hybrids (presence of chromosome/blot hybridization)

Mouse chromosome	Concordant +/+	Concordant −/−	Concordant Total	Discordant +/−	Discordant −/+	Discordant Total
1	1	9	10	9	1	10
2	1	9	10	9	0	9
3	1	10	11	9	0	9
4	1	9	10	11	0	11
5	1	12	13	6	1	7
6	1	7	8	12	0	12
7	1	5	6	14	0	14
8	1	12	13	7	0	7
9	1	6	7	12	1	13
10	1	9	10	10	0	10
11	1	18	19	0	0	0
12	1	3	4	16	0	16
13	0	3	3	4	1	5
14	1	16	17	2	1	3
15	1	3	4	5	0	5
16	0	4	4	4	1	5
17	1	7	8	12	0	12
18	1	13	14	2	0	2
19	1	7	8	10	0	10
X	1	2	3	12	0	12

Summary of the blot hybridization results for 21 mouse×hamster hybrid cell line DNAs probed with the *Hox-2* DNA fragment (see Fig. 1*A*). The presence or absence of mouse chromosome 11 is concordant with the presence or absence of the *Hox-2* diagnostic mouse bands.

other more weakly hybridizing bands are seen in both the mouse and hamster genomes (Fig. 1*B*). Scoring the hybrid cell lines for the *Hox-2* mouse-specific fragments assigns this genomic locus to mouse chromosome 11. Table 1 summarizes the data, showing no discordancies either for the presence or absence of chromosome 11 in the hybrids or for the presence or absence of the diagnostic murine restriction fragments.

The human homoeo box sequences, *Hu-1* and *Hu-2*, were regionally mapped by *in situ* hybridization to human metaphase chromosomes[10,11]. The chromosomes were identified by G-banding before hybridization. After hybridization and autoradiography, the distribution of silver grains over 73 previously photographed chromosome spreads was plotted on a histogram (Fig. 2*A*) in which a standard idiogram of the haploid human karyotype was divided into 238 units scaled to the average size of a silver grain (0.35 μm). Of 712 grains assigned to chromosomes, 140 (20%) were specifically localized to 17q11–q22, which represents 0.8% of the haploid genome length. Statistical evaluation (by Poisson distribution) of the number of grains per unit chromosome length confirmed a highly significant concentration of label within this region ($P \ll 0.001$). Figure 2*B* shows a representative chromosome spread hybridized with the *Hu-1* and *Hu-2* probes. Analysis of a further 24 labelled chromosomes 17 from 30 metaphase spreads (Fig. 2*C*) illustrates the localization of the *Hu-1* and *Hu-2* homoeo box probes to 17q11–q22.

Comparative mapping between mouse and human has revealed conservation of syntenic linkage associations for several chromosomes[12–14]. Here we present data indicating that two mouse and human homoeo box loci map to evolutionarily related chromosome regions in these species. The human *Hu-1* and *Hu-2* clones have been regionally localized to 17q11–q22; also present at this site are the pro-α1(I) collagen gene[15,16]; the thymidine kinase (TK) and galactokinase (GALK) genes, which have been shown to be tightly linked (within 1.2 centimorgans)[17–19]; a cluster of genes for growth-regulating hormones, including growth hormone and CSH[20,21]; and the cellular homologue of the *erb-A1* oncogene, which is believed to enhance the transforming potential of leukaemogenic cells[22,23]. The mouse *Hox-2* locus has been assigned to chromosome 11; the murine TK and GALK genes have previously been mapped 71 centimorgans from the centromere of this chromosome[24,25]. As

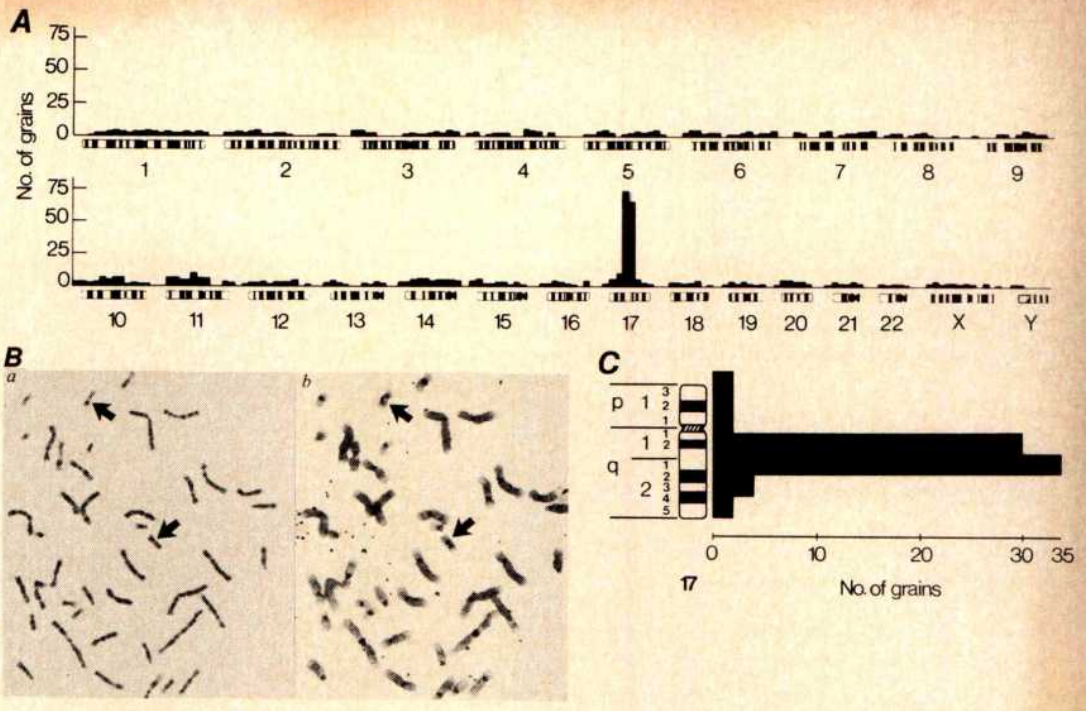


Fig. 2 Regional localization of the human *Hu-1* and *Hu-2* homoeo box sequences. *A*, Distribution of autoradiographic silver grains over 73 chromosome spreads after *in situ* hybridization with ^{125}I-labelled *Hu-1* and *Hu-2*. The data were plotted on a histogram representing the haploid human karyotype. If 712 grains appearing on chromosomes, 140 (20%) were localized within the region 17q11–q22. *B*, G-banded chromosome spread (JS cell line) before (*a*) and after (*b*) hybridization. The arrows indicate *Hu-1* and *Hu-2* signal on chromosome 17. *C*, Distribution of silver grains on labelled chromosomes 17 from 30 metaphase spreads, showing localization of the *Hu-1* and *Hu-2* homoeo box sequences to 17q11–q22.

Methods. Human metaphase chromosome spreads were prepared by standard techniques from cultures of karyotypically normal Epstein–Barr virus-transformed lymphoblastoid cell lines (JS, DB and PM). *Hu-1* and *Hu-2*, containing 1.7- and 2.1-kb human DNA inserts, respectively[8], were mixed 1:1 and nick-translated with ^{125}I-dCTP (2,000 Ci mmol^{-1}; Amersham) to a specific activity of 6.4×10^8 d.p.m. μg^{-1} (ref. 31). Unincorporated nucleoside triphosphates were removed by CF11 cellulose chromatography[32]. Chromosome preparations were hybridized with ^{125}I-labelled *Hu-1* and *Hu-2* probe mix (0.15 μg ml^{-1}) at 37 °C for 16 h and autoradiographed for 7–21 days as described previously[10,11].

linkage of the genes for TK and GALK has been conserved in both species, the mouse *Hox-2* locus probably maps close to these genes. These data also suggest that the *Hox-2* locus in the mouse is homologous to the genomic domain containing the human homoeo box regions *Hu-1* and *Hu-2*. It will be interesting to determine whether the murine *erb-1* gene which has been assigned to mouse chromosome 11, maps within this linkage group also.

Genetic mapping studies can suggest or eliminate possible allelism of the homoeo box loci with genomic loci known to affect mammalian development. In the mouse, a gene not ruled out by such comparison is *tail-short* which maps 65 centimorgans from the centromere of mouse chromosome 11 (ref. 26). Homozygotes for *tail-short* do not survive beyond day $5\frac{1}{2}$ of gestation; heterozygotes have skeletal abnormalities which include vertebral fusions, bilateral asymmetry of limb length, triphalangy and an additional pair of ribs. Fine-structure mapping of the *Hox-2* locus using restriction fragment length polymorphisms and recombinant inbred strains of mice could indicate a coincident map position and justify investigation at the molecular level of the relation between the *Hox-2* locus and the *tail-short* mutation. A similar rationale has been proposed to test for allelism between the *Hox-1* locus and hypodactyly, another mutation affecting morphogenesis, which both map to the same region on chromosome 6 (ref. 9). While the function of the mouse homoeo box loci remains speculative, the recent observation that they are transcriptionally active only at specific stages of mouse embryogenesis (C.P.H., Awgulewitsch and F.H.R., in preparation) indicates that their expression is subject to strict temporal regulation, as expected for a gene which in turn modulates morphogenetic patterns. Dysmorphic syndromes in humans include dyssegmental dysplasias[27] and anispondylic comptomicromelic dwarfism (dyssegmental dwarfism)[28,29], which are neonatally lethal genetic lesions characterized by vertebral segmentation defects. Recent evidence[30] suggests an association between dyssegmental dwarfism and defects in the metabolism of α1 collagen, the gene for which maps to the same region of chromosome 17 as the homoeo box loci reported here[15,16]. As these cases represent rare occurrences in the general population, there is insufficient genetic data to indicate involvement of homoeo box loci at present. To obtain a better understanding of the function of homoeo box regions during development, studies are under way to compare the temporal and spatial expression of homoeo box sequences during embryogenesis in mouse and man, by blot hybridization to mRNA purified from embryos obtained at progressive stages of gestation and by *in situ* hybridization to endogenous mRNAs in embryo tissue sections. If these genetic loci behave as predicted by analogy to the *Drosophila* homoeotic genes, such data will provide insights into the molecular events controlling mammalian development.

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1. McGinnis, W., Levine, M., Hafen, E., Kuroiwa, A. & Gehring, W. J. *Nature* **308**, 428–433 (1984).
2. McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. & Gehring, W. J. *Cell* **37**, 403–408 (1984).
3. Hafen, E., Levine, M., Garber, R. L. & Gehring, W. J. *EMBO J.* **2**, 617–623 (1983).
4. Hafen, E., Kuroiwa, A. & Gehring, W. J. *Cell* **37**, 833–841 (1984).
5. Carrasco, A. E., McGinnis, W., Gehring, W. J. & DeRobertis, E. M. *Cell* **37**, 409–414 (1984).
6. Shepherd, J. C. W., McGinnis, W., Carrasco, A. E., DeRobertis, E. M. & Gehring, W. J. *Nature* **310**, 70–71 (1984).
7. Laughon, A. & Scott, M. P. *Nature* **310**, 25–31 (1984).
8. Levine, M., Rubin, G. M. & Tjian, R. *Cell* **38**, 667–673 (1984).
9. McGinnis, W., Hart, C. P., Gehring, W. J. & Ruddle, F. H. *Cell* **38**, 675–680 (1984).
10. Rabin, M. *et al. Cytogenet. Cell Genet.* **38**, 70–72 (1984).
11. Barker, P. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 5826–5830 (1984).
12. Francke, U. & Taggart, R. T. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3595–3599 (1980).
13. Roderick, T. H. *et al. Cytogenet. Cell Genet.* **37**, 312–316 (1984).
14. Kamarck, M. E., Barker, P. E., Miller, R. L. & Ruddle, F. H. *Expl Cell Res.* **152**, 1–14 (1984).
15. Church, R. L., SundarRaj, N. & McDougall, J. K. *Cytogenet. Cell Genet.* **27**, 24–30 (1980).
16. Huerre, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6627–6630 (1982).
17. McDougall, J. K., Kucherlapati, R. & Ruddle, F. H. *Nature new Biol.* **245**, 172–175 (1973).
18. Elsevier, S. M. *et al. Nature* **251**, 633–636 (1974).
19. Gross, S. *Cytogenet. Cell Genet.* **25**, 161–164 (1979).
20. George, D. L., Phillips, J. A. III, Francke, U. & Seeburg, P. H. *Hum. Genet.* **57**, 138–141 (1981).

21. Harper, M. E., Barrera-Saldana, H. A. & Saunders, G. F. *Am. J. hum. Genet.* **34**, 227-234 (1982).
22. Zabel, B. U., Fournier, R. E. K., Lalley, P. A., Naylor, S. L. & Sakaguchi, A. Y. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4874-4878 (1984).
23. Frykberg, L. *et al. Cell* **32**, 227-238 (1983).
24. Kozak, C. A. & Ruddle, F. H. *Somat. Cell Genet.* **3**, 121-133 (1977).
25. McBreen, P., Orkwiszewski, K. G., Chern, C. J., Mellman, W. J. & Croce, C. M. *Cytogenet. Cell Genet.* **19**, 7-13 (1977).
26. Green, M. C. *Genetic Variants and Strains of the Laboratory Mouse* (Fischer, New York, 1981).
27. Aleck, K. *et al. Am. J. hum. Genet.* **36**, 41S (1984).
28. Handmaker, S. D., Robinson, L. D., Campbell, J. A., Chinwah, O. & Gorlin, R. J. *Birth Defects orig. Artic. Ser.* **13**, (3D), 79-90 (1977).
29. Gorlin, R. J. & Langer, L. O. Jr *Birth Defects orig. Artic Ser.* **14**, (6B), 193-197 (1978).
30. Svejcar, J. *Clin. Genet.* **23**, 369-375 (1983).
31. Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).
32. Szabo, P., Elder, R., Steffensen, D. M. & Uhlenbeck, O. C. *J. molec. Biol.* **115**, 539-563(1977).

A *Drosophila* genomic sequence with homology to human epidermal growth factor receptor

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Vertebrate genomes contain an extensive family of genes possessing varying degrees of homology to the v-*src* oncogene. Most *src*-related proteins identified to date are intracellular and membrane-associated, although some are transmembrane proteins and function as receptors for peptide growth factors[1-6]. Three *Drosophila* gene sequences related to the v-*src* gene have been identified, each exhibiting a high degree of homology to one or more of the *src*-family members encoding an intracellular protein[7-9]. We have isolated a panel of cloned *Drosophila* sequences exhibiting weak v-*src* hybridization and were interested to determine whether any members of this group represented homologues of additional known *src*-family genes, especially those functioning as growth factor receptors. As we report here, four of these clones, representing overlapping portions of the same genomic segment, hybridized preferentially with the v-*erb-B* oncogene and were further characterized. The deduced amino-acid sequence from a portion of this *Drosophila* genomic segment is 77% homologous to the kinase domain of human epidermal growth factor (EGF) receptor, a substantially greater degree of homology than was observed with any other known *src*-family member. By hybridization with a human EGF receptor complementary DNA clone probe[10], we demonstrate that the same genomic segment showing homology with the kinase domain also contains sequences related to the extracellular domain of the EGF receptor gene.

By screening a *Drosophila* genomic library using hybridization with v-*src* sequences, two types of clones were isolated, exhibiting respectively strong and weak homology with v-*src*. Each of the clones in the former group represented one of the three major *Drosophila src*-related sequences characterized previously[7-9]. To exclude the possibility that the members of the group showing weak homology to v-*src* were repeat isolates of the previously characterized *Drosophila src*-related sequences, DNA from these clones was hybridized in stringent conditions with probes representing each of the three major *Drosophila src*-related genes; none of the clones reacted with any of these sequences. As the *src* gene family in vertebrates contains many divergent members, we wished to test whether the clones showing low homology to v-*src* contained other known vertebrate *src*-family sequences. The first *src*-family oncogene chosen for secondary screening of these clones was v-*erb-B*, which contains a portion of the avian EGF receptor gene[6]. DNA from each of the 11 clones in the low-homology group was tested by Southern blot[11] hybridization with the 0.56-kilobase (kb) *Bam*HI segment of v-*erb-B*[12]. This portion of the v-*erb-B* sequence encodes the kinase domain, the most highly conserved region of *src*-family genes. A variable-sized *Eco*RI segment and a 1-kb *Taq*I fragment from each of the clones Dsrc13, Dsrc16, Dsrc25 and Dsrc42 hybridized more strongly with the v-*erb-B* probe than did DNA from the other seven clones. The 1-kb *Taq*I fragment also hybridized weakly but reproducibly with the 0.54-kb *Bam*HI/*Eco*RI segment of v-*erb-B*, adjacent to and downstream from the 0.56-kb *Bam*HI fragment of v-*erb-B*[4] (Fig. 1).

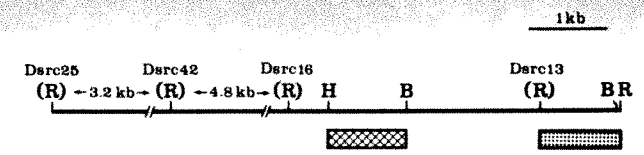


Fig. 1 Restriction endonuclease cleavage map of clones Dsrc13 and Dsrc16. R, *Eco*RI sites; B, *Bam*HI; H, *Hin*dIII. The v-*erb-B*-complementary *Eco*RI segments of Dsrc13 and Dsrc16 were subcloned into pUC8 for sequencing and restriction mapping. The *Eco*RI sites in parentheses are not genomic sites but are a result of the introduction of linkers during cloning. The dotted box below the map indicates the region that hybridized with v-*erb-B* *Bam*HI and *Bam*HI/*Eco*RI probes, while the cross-hatched box indicates the region that hybridized with the *Eco*RI/*Nae*I probe specific for the extracellular domain of the human EGF receptor. Hybridizations with the heterologous probes were conducted as follows. Southern blots were pre-hybridized for 3 h at 60 °C in 4×SET (1×SET is 0.15 M NaCl, 0.03 M Tris-HCl *p*H 8.0, 2 mM EDTA), 10×Denhardt's solution[18], 0.1% SDS and 0.1% sodium PP_i and for an additional hour in the same solution containing denatured calf thymus DNA (Sigma) at 50 μg ml^{-1}. After overnight hybridization in the latter solution in the presence of denatured ^{32}P-labelled probe, the filters were washed according to the following schedule: 1 h at 60 °C in the hybridization solution; three 45-min intervals at 60 °C in 3×SET, 0.1% SDS and 0.1% sodium PP_i; 45 min at 60 °C in 0.3×SET, 0.1% sodium PP_i, 0.1% SDS and a final wash in 4×SET at room temperature.

Because these clones were isolated by low-stringency hybridization, it was important to determine the nucleotide sequence of a portion of the *Drosophila* DNA so as to demonstrate significant structural homology with the vertebrate EGF receptor gene. The 1-kb *Eco*RI segment of Dsrc13 which hybridized with v-*erb-B* probes was therefore subcloned into plasmid pUC8 and further subcloned into phage M13 mp8. Figure 2 shows the nucleotide sequence and the deduced amino-acid sequence of a portion of the Dsrc13 clone. The *Drosophila* sequence begins at the left end of Dsrc13 (Fig. 1) and corresponds to the cDNA sequence of human EGF receptor[10] at positions 2,383–2,742. This region represents the first 60% of the v-*erb-B* *Bam*HI fragment used as hybridization probe to identify the *Drosophila* clones.

There are several points of note about the sequence data. First, overall there is 64% nucleotide sequence homology between the two sequences. The longest region of extensive homology, 70%, is from position 166 to the end of the sequence. As the sequence of the *Drosophila* clone further downstream exhibits less homology with the EGF receptor sequence (data not shown), it is apparent that the region shown was responsible for the observed hybridization with the v-*erb-B* *Bam*HI probe. Second, this region of the avian c-*erb-B* gene is interrupted by at least two introns totalling ~4,600 base pairs[13]. As the *Drosophila* DNA sequence and human cDNA sequences are co-linear, this region of the *Drosophila* gene lacks the two introns present in the avian gene. The structure of the analogous region of the human EGF receptor gene has not been documented. Third, at the amino-acid level, there is 62% homology between the *Drosophila* and human sequences. However, at position 166–360, the amino-acid sequence homology is 77%. It is perhaps noteworthy that the lysine residue at position 295 in the v-*src* protein, which is highly conserved in *src*-family proteins, is also conserved in the *Drosophila* sequence (encoded by nucleotides 37–39, Fig. 2). The analogous amino acid in cyclic AMP-dependent protein kinase is thought to be located in the nucleotide-binding site of the enzyme[14-16].

Because the kinase domain of *src*-family genes is highly conserved, it was important to demonstrate that the homology between the EGF receptor sequence and the Dsrc13 sequence

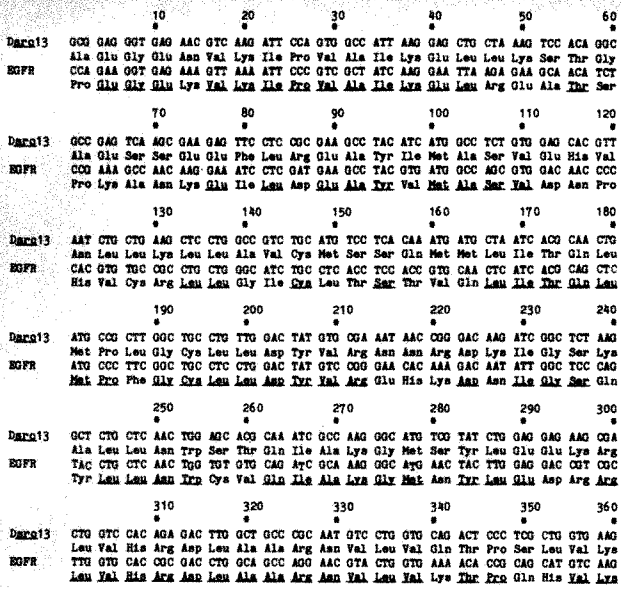


Fig. 2 Nucleotide sequence of a portion of the subcloned *Eco*RI segment from Dsrc13 and the deduced amino-acid sequence. Sequencing was performed by the chain-terminator method[19] using M13 mp8 templates generated by cleavage with *Eco*RI, *Msp*I or *Sau*3A. The sequences were aligned and translated using computer programs[20]. The sequence of the human EGF receptor was taken from ref. 10. Position 1 in the *Drosophila* sequence corresponds to nucleotide 2,383 in the human sequence. Underlined amino acids in the human sequence indicate amino acids conserved in the *Drosophila* sequence.

was significantly higher than that between other *src*-family genes. For this, we selected the region of the *Drosophila* sequence exhibiting the greatest homology with the EGF receptor sequence, nucleotides 166–360. Figure 3 compares the *Drosophila* amino-acid sequence with the sequences of other *src*-family genes. The finding that the homology between the *Drosophila* sequence and the EGF receptor exceeds the next highest comparison by 25% indicates that the Dsrc13 sequence is significantly more closely related to the kinase domain of the EGF receptor than to other known *src*-family genes. Comparisons of the Dsrc13 sequence with v-*raf*, v-*mos* and v-*fgr* are not shown, as the homologies were even lower than those shown in Fig. 3.

The relationship of the cloned *Drosophila* sequences to genomic sequences was defined by a series of Southern blot hybridizations. As noted above, *Eco*RI segments of varying sizes were detected in these clones by v-*erb-B* hybridization, the largest being >12 kb in clone Dsrc25. This suggested that the *Eco*RI site terminating the left-hand end of clone Dsrc13 (as well as Dsrc16 and Dsrc42) was not a genomic site and therefore Dsrc13 and v-*erb-B* probes should hybridize to a genomic *Eco*RI fragment of 12 kb or larger. In addition, as the v-*erb-B* probe detected a *Taq*I fragment common to each of the clones, a similar *Taq*I fragment should be present in genomic DNA. When *Drosophila* genomic DNA restricted with either *Eco*RI or *Taq*I was hybridized in low-stringency conditions with the v-*erb-B Bam*HI probe (Fig. 4*a*), two *Eco*RI segments of 22 and 13.5 kb and two *Taq*I segments of 1 and 0.5 kb were detected. The two *Eco*RI segments hybridized with similar efficiency, while the smaller *Taq*I segment hybridized weakly and is not visible as reproduced here. The 1-kb *Taq*I fragment was equivalent in size to the *Taq*I fragment from each of the Dsrc13, 16, 25 and 42 clones which was also detected by v-*erb-B* hybridization. The same genomic blot was subsequently hybridized with a probe from the subcloned *Eco*RI segment of Dsrc13 (Fig. 1) in high-stringency (Fig. 4*b*) and low-stringency conditions (Fig. 4*c*). In high-stringency hybridization conditions, the Dsrc13 probe detected only the 22-kb *Eco*RI segment and the 1-kb *Taq*I segment. Thus, the larger of the two genomic *Eco*RI segments contains the sequences represented by the overlapping set of clones that we have identified by v-*erb-B* hybridization. In low-stringency hybridization conditions, the Dsrc13 probe still hybridized primarily with the larger of the *Eco*RI and *Taq*I bands, although weak hybridization to the v-*erb-B*-complementary 13.5-kb *Eco*RI segment and at least one additional *Eco*RI segment was observed. These results suggest that there is a single-copy genomic segment represented by the set of overlapping clones Dsrc13, 16, 42 and 25 identified by hybridization with v-*src* and v-*erb-B* probes. The presence of additional genomic sequences showing homology to v-*erb-B* is suggested by the smaller *Eco*RI segments that hybridized with the v-*erb-B* and Dsrc13 probes in less stringent conditions. We believe that some of the additional genomic sequences detected by v-*erb-B* hybridization represent *Drosophila src*-family sequences related to the kinase domain of the v-*erb-B* oncogene. Confirmation of this hypothesis awaits the isolation of the remainder of the v-*erb-B*-complementary genomic segments.

Although the above results documented a degree of homology between Dsrc13 and the cytoplasmic domain of the human EGF receptor, we could not conclude that the *Drosophila* sequences represented a true homologue of the vertebrate gene. In addition to the kinase domain, the EGF receptor contains a short transmembrane domain and a large extracellular domain that is responsible for EGF binding[10], and it was important to determine whether other portions of the vertebrate gene were represented on one or more of our *Drosophila* clones. An *Eco*RI/*Nae*I fragment from the human EGF receptor cDNA clone was isolated as a hybridization probe specific for the extracellular domain of EGF receptor; this fragment starts at position 293 from the extreme 5′ end of the amino-acid-coding region and stops short of the transmembrane domain by 100 nucleotides[10]. Hybridization of clones Dsrc16, 42 and 25 with the *Eco*RI/*Nae*I probe revealed the same *Eco*RI segments that hybridized with the v-*erb-B Bam*HI probe (data not shown, see Fig. 1). These *Eco*RI segments vary in size due to the introduction of *Eco*RI linker sites during construction of the library[17]. DNA from clone Dsrc13 did not hybridize with the *Eco*RI/*Nae*I probe, a not unexpected finding considering the sequence and arrangement of this clone. To characterize further the homology of the *Eco*RI/*Nae*I probe to *Drosophila* sequences, the pattern of hybridization to cloned and genomic sequences was determined. In the cloned DNA, *Eco*RI/*Nae*I probe hybridization was limited to a 1-kb *Hin*dIII/*Bam*HI segment (Fig. 4*d*). The pattern of *Eco*RI/*Nae*I probe hybridization to genomic DNA

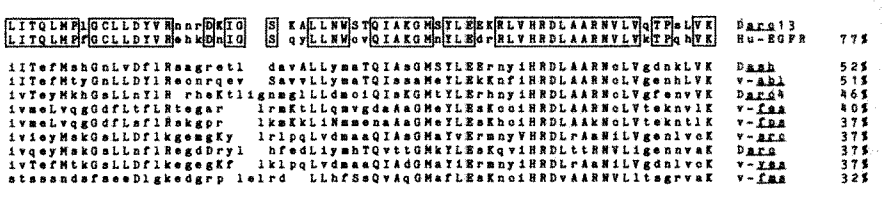


Fig. 3 Alignments of deduced amino-acid sequences of Dsrc13, EGF receptor and other *src*-family members. The sequence of Dsrc13 for amino acids 56–120 was aligned with the analogous region of various *src*-related proteins. The gaps in some of the sequences were introduced manually to achieve maximal alignment of the most highly conserved regions. Only the matches between Dsrc13 and EGF receptor are boxed. Positions in the other sequences that match the Dsrc13 sequence are shown in capital letters. Lower-case letters in the Dsrc13 sequence indicate that no other sequence matches at that position. The numbers on the right-hand side indicate the homology of that sequence with Dsrc13. The sources of the sequence data were as follows: D*ash*, ref. 7; v-*abl*, ref. 7; Dsrc4, ref. 9; v-*fes*, ref. 21; v-*fps*, ref. 22; v-*src*, ref. 23; Dsrc, ref. 7; v-*yes*, ref. 24; v-*fms*, ref. 25.

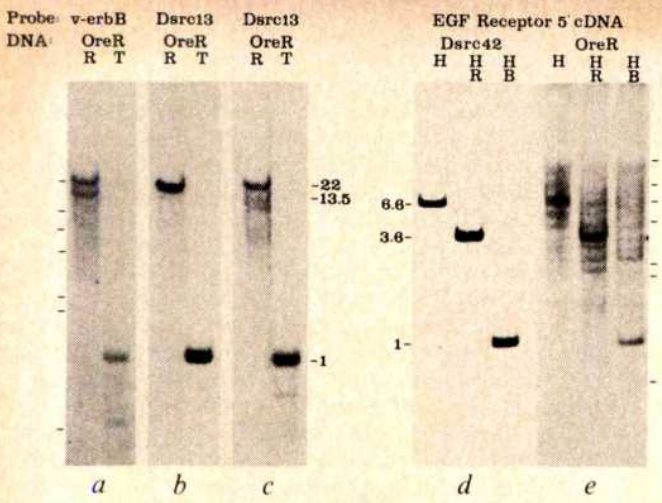


Fig. 4 Southern blot hybridization analysis of cloned and genomic sequences. In *a–c*, Southern blots of Oregon R genomic DNA cleaved with either *Eco*RI (R) or *Taq*I (T) were hybridized with the v-*erb-B* *Bam*HI fragment probe or the Dsrc13 *Eco*RI fragment probe. In *d* and *e*, respectively, Southern blots of whole phage DNA from the Dsrc42 clone (see Fig. 1) or Oregon R genomic DNA cleaved with *Hin*dIII (H), *Hin*dIII plus *Eco*RI (HR) or *Hin*dIII plus *Bam*HI (HB) were hybridized with the *Eco*RI/*Nae*I probe specific for the extracellular domain of the human EGF receptor cDNA clone. With the exception of *b*, hybridizations were conducted as described in Fig. 1 legend; for *b*, the hybridization conditions were identical except that the temperature of hybridization was 67 °C. The dashes on the left and right sides of the figure indicate the positions of λ DNA cleaved with *Hin*dIII. The approximate sizes of the hybridizing DNA fragments noted in the text are also shown.

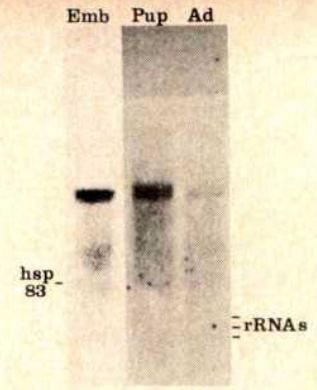


Fig. 5 RNA expression of Dsrc13 sequences. Approximately 5-μg samples of poly(A)-containing RNA from an 18-h collection of *Drosophila* embryos (Emb), mid-metamorphic stage animals (Pup) and adult flies (Ad) were electrophoresed through formaldehyde/agarose gels, blotted and hybridized with Dsrc13 probe as described previously[26]. The positions of the rRNA species (2 kb, 1.9 kb and 1.7 kb) were determined by staining of the nitrocellulose filter. The position of the *hsp83* mRNA (3.05 kb) was determined by hybridization with cloned *hsp83* gene sequences[27].

was essentially the same as in the cloned DNA (Fig. 4*e*). In each digest, the unique band of hybridization in the cloned DNA corresponded in size to the major band of hybridization in genomic DNA. We therefore conclude that the major genomic sequence detected by hybridization with the extracellular domain probe of EGF receptor is located on the same cloned DNA segment that has homology with the kinase domain of the EGF receptor. Additional nucleotide sequencing experiments are in progress to confirm the identity of the sequences in the region of our *Drosophila* clones which hybridize with the *Eco*RI/*Nae*I probe. Hybridization of the *Eco*RI/*Nae*I probe to additional genomic sequences was also observed (Fig. 4*e*), but their nature is unknown. However, the v-*erb-B* probe also detected genomic sequences not present on our cloned DNA segments (Fig. 4*a*). Taken together, these results suggest that other genomic sequences related to both domains of the EGF receptor may be responsible for the observed hybridization.

As the Dsrc13 probe contains sequences from an amino-acid-coding region and does not contain repeated sequences detectable in high-stringency hybridization conditions (Fig. 4*b*), this probe was used to test for RNA expression of the putative EGF receptor gene, and readily revealed a poly(A)-containing transcript of 6 kb in embryos, pupae and adults (Fig. 5). In pupal RNA, this band seems to be a closely spaced doublet. These results suggest that the sequences exemplified by the Dsrc13 clone represent a functional *Drosophila* gene.

In vertebrate cells, the mitogenic activity of EGF is transmitted through the EGF receptor. Because of its different developmental programme, the study of these sequences in *Drosophila* offers a different perspective of EGF receptor function. For the first 2 h of *Drosophila* development, there are virtually continuous rounds of chromosome and nuclear divisions although there are no cells and no cell divisions. At the blastoderm stage, the fate of cells is determined such that some cells, the imaginal cells, will divide mitotically until the adult is formed during metamorphosis. Other cells that give rise to larval tissues will not divide during the 4 days of larval life although chromosome duplication does continue. Thus, there are fluctuations in the mitotic states of cells in *Drosophila* that have no counterpart in vertebrate systems. We plan to exploit these differences to enhance our understanding of EGF receptor structure and function and its involvement in *Drosophila* development.

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Note added in proof: Nucleotide sequence analysis of the Dsrc16 clone centred around the leftmost *Bam*HI site (Fig. 1) has revealed a region having amino-acid sequence homology with the extracellular domain of the human EGF receptor. Within a 260-base pair region, the overall amino-acid sequence homology is ~32% but includes a region in which 9 of 11 amino acids match (amino acids 373–383 in the human sequence[10]).

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1. Bishop, J. M. *A. Rev. Biochem.* **52,** 301–354 (1983).
2. Buhrow, S. A., Cohen, S. & Stavros, J. V. *J. biol. Chem.* **257,** 4019–4022 (1982).
3. Ek, B., Westermark, B., Wasteson, A. & Heldin, C.-H. *Nature* **295,** 419–420 (1982).
4. Nishimura, J., Huang, J. S. & Deuel, T. F. *Proc. natn. Acad. Sci. U.S.A.* **79,** 4303–4307 (1982).
5. Cooper, J. A., Bowen-Pope, D. F., Raines, E., Ross, R. & Hunter, T. *Cell* **31,** 263–273 (1982).
6. Downward, J. *et al. Nature* **307,** 521–527 (1984).
7. Hoffmann, M. F., Fresco, L. D., Hoffman-Falk & Shilo, B.-Z. *Cell* **35,** 393–401 (1983).
8. Simon, M. A., Kornberg, T. B. & Bishop, J. M. *Nature* **302,** 837–839 (1983).
9. Wadsworth, S. C., Madhavan, K. & Bilodeau-Wentworth, D. *Nucleic Acids Res.* (submitted).
10. Ullrich, A. *et al. Nature* **309,** 418–425 (1984).
11. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
12. Yamamoto, T. *et al. Cell* **35,** 71–78 (1983).
13. Vennstrom, B. & Bishop, J. M. *Cell* **28,** 135–143 (1982).
14. Barker, W. C. & Dayhoff, M. O. *Proc. natn. Acad. Sci. U.S.A.* **79,** 2836–2839 (1982).
15. Shozo, S. *et al. Proc. natn. Accd. Sci. U.S.A.* **78,** 848–851 (1981).
16. Zoller, M. J. & Taylor, S. S. *J. biol. Chem.* **254,** 8363–8368 (1979).
17. Maniatis, T. *et al. Cell* **15,** 687–701 (1978).
18. Denhardt, D. T. *Biochem. biophys. Res. Commun.* **23,** 641–646 (1966).
19. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
20. Pustell, J. & Kafatos, F. C. *Nucleic Acids Res.* **10,** 4765–4782 (1982).
21. Hampe, A., Laprevotte, I., Gailbert, F., Fedele, L. A. & Sherr, C. J. *Cell* **30,** 775–785 (1982).
22. Shibuya, M. & Hanafusa, H. *Cell* **30,** 787–795 (1982).
23. Schwartz, D. E., Tizard, R. & Gilbert, W. *Cell* **32,** 853–869 (1983).
24. Kitamura, N., Kitamura, A., Toyoshima, K., Hirayama, Y. & Yoshida, M. *Nature* **297,** 205–208 (1982).
25. Hampe, A., Gobet, M., Sherr, C. J. & Gailbert, F. *Proc. natn. Acad. Sci. U.S.A.* **81,** 85–89 (1984).
26. Madhavan, K., Bilodeau-Wentworth, D. & Wadsworth, S. C. *Molec. cell. Biol.* **5,** 7–16 (1985).
27. Holmgren, R., Livak, K., Morimoto, R., Freund, R. & Meselson, M. *Cell* **18,** 1359–1370 (1979).

Investigation of genetic linkage between myosin and actin genes using an interspecific mouse back-cross

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The introduction of cloned probes to follow the segregation of DNA restriction fragment length polymorphisms (RFLPs) has led to a revival of mendelian genetics in attempts to map the human genome[1]. In the mouse, however, it has often proved difficult to detect an RFLP with a DNA probe between different inbred strains of the laboratory mouse. To circumvent this problem, we have used two species, *Mus musculus domesticus* and *Mus spretus*[2] which interact as sympatric species[3] but can be interbred under laboratory conditions[4]. Because of the relative evolutionary distance between these species, they exhibit polymorphism at many more loci than do different strains of the usual *M. m. domesticus* laboratory mouse[2]. This is also observed at the DNA level when the sizes of restriction fragments encoding a specific gene are compared[5]. We have used these RFLPs between *M. m. domesticus* and *M. spretus* to follow the segregation of genes encoding different isoforms of myosin alkali light chains in the backcross progeny between these species and to compare this with that of other contractile protein genes. No linkage between these genes was observed.

We have characterized several DNA sequences which serve as specific probes for genes encoding different isoforms of myosin alkali light chains in the mouse, including the isoforms of fast skeletal muscle ($MLC1_F/MLC3_F$)[5], cardiac ventricular muscle ($MLC1_V$), which is also the major isoform expressed in slow skeletal muscle ($MLC1_S$)[6], and cardiac atrial muscle ($MLC1_A$), also expressed in fetal skeletal and ventricular muscle ($MLC1_{emb}$)[7,8]. The two light chains, $MLC1_F$ and $MLC3_F$, are encoded by a single functional locus, from which a processed pseudogene has been generated in some mouse species (for example, *Mus musculus*); this is absent from *M. spretus*[5]. The other isoforms are encoded by unique loci[6,8]. These probes reveal fragments of different sizes in the DNA of inbred lines of *M. spretus* (SPE/Pas) and of *M. musculus* (DBA/2) on restriction with many different enzymes, which thus characterize allelic forms of the genes.

The segregation of the myosin light-chain (MLC) alleles in 42 offspring from a back-cross is presented in Table 1 and analysed in Table 2. An example of such an experiment with the $MLC1_A$ ($MLC1_{emb}$) gene is shown in Fig. 1. There is no co-segregation of the genes encoding $MLC1_F/MLC3_F$, $MLC1_A$ ($MLC1_{emb}$) or $MLC1_V$ ($MLC1_S$), thus, there is no genetic linkage. In this backcross analysis, linkage would have been detected for genes up to 30 centimorgans (cM) apart, with a 99% confidence limit[9]. Twenty-one previously localized polymorphic markers[10], covering about half of the mouse genome, have been analysed for their segregation in the same back-cross. Comparison with the segregation of the myosin light-chain genes demonstrates linkage of the gene for $MLC1_F/MLC3_F$ to *Idh-1* (chromosome 1), for $MLC1_V$ ($MLC1_S$) to *Es-14* (chromosome 9) and for $MLC1_A$ ($MLC1_{emb}$) to *Es-3* (chromosome 11). Therefore, these myosin light-chain genes are located on different mouse chromosomes.

The localization of the $MLC1_F/MLC3_F$ gene was confirmed by the analysis of B × D recombinant inbred strains derived from

Table 1 Segregation of the contractile protein genes and of biochemical markers in the back-cross

	Animal no.																					Chromosomal assignments
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	of markers
$MLC1_F/MLC3_F$	−	+	+	+	+	−	−	−	−	+	+	−	−	+	+	+	+	−	−	−	−	
Idh-1	−	+	+	+	+	−	−	−	−	+	+	−	−	+	+	+	+	−	−	ND	−	1
$MLC1_V$	−	−	+	−	+	+	+	+	−	+	+	−	−	−	−	−	+	+	−	+	+	
Es-14	−	−	+	−	+	+	+	+	−	+	+	−	−	−	−	−	+	+	−	ND	+	9
$MLC1_A$	−	+	−	−	−	+	−	−	+	−	+	+	+	+	−	+	−	+	+	−	+	
Es-3	+	+	−	−	−	+	−	−	+	−	+	+	+	+	−	+	−	+	+	ND	+	11
MHC_F	−	+	+	−	+	−	+	−	+	+	+	+	+	−	−	+	−	+	+	−	+	
Hba	+	−	+	ND	+	−	+	−	+	+	+	−	+	−	ND	ND	−	+	−	−	ND	11
α-Cardiac actin	+	−	−	−	+	−	+	−	+	−	+	+	−	+	+	+	−	−	+	−	+	
α-Skeletal actin	+	+	−	+	−	−	+	−	+	−	+	+	−	−	−	+	+	−	+	−	+	

	Animal no.																					Chromosomal assignments
Locus	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	of markers
$MLC1_F/MLC3_F$	+	−	+	+	−	−	+	−	+	+	−	+	−	+	+	−	−	+	+	−	−	
Idh-1	+	−	+	−	−	−	+	+	+	+	−	+	−	+	+	−	−	ND	ND	ND	ND	1
$MLC1_V$	−	−	−	−	+	−	−	−	+	−	+	+	−	+	−	−	+	+	−	+	+	
Es-14	−	−	−	−	+	−	−	−	+	−	+	+	−	+	−	−	+	ND	ND	ND	ND	9
$MLC1_A$	−	+	+	+	+	−	+	−	−	+	−	−	−	+	+	+	−	−	−	+	−	
Es-3	−	+	+	−	+	−	+	+	−	+	−	−	−	+	+	+	−	ND	ND	ND	ND	11
MHC_F	+	+	+	+	−	−	+	+	−	+	−	−	−	+	+	+	−	+	−	+	+	
Hba	+	ND	ND	+	−	+	−	ND	ND	ND	−	−	−	+	−	−	+	+	+	ND	ND	11
α-Cardiac actin	+	−	+	+	+	−	−	−	−	+	+	+	−	+	+	+	−	−	+	−	+	
α-Skeletal actin	−	−	+	−	+	+	−	−	+	−	−	+	−	+	−	+	−	−	−	+	−	

The animals are scored as homozygous for the *musculus* alleles (−) or heterozygous (+). ND, not determined. Animals 1-20 are females, 21-42 are males. *Idh-1*, *Es-3* and *Es-14* were analysed as described in ref. 20. The segregation of *Hba* was analysed using a *Hin*dIII RFLP detected with an α-globin cDNA[21]; see Fig. 1. Similarly, the $MLC1_F/MLC3_F$ gene was analysed using *Eco*RI or *Bcl*I (blot washing at 45 °C), the $MLC1_V$ ($MLC1_S$) gene using *Bam*HI (blot washing at 45 °C), the MHC_F gene using *Bgl*I (blot washing at 72 °C), the α-cardiac actin gene using *Bcl*I or *Sac*I (blot washing at 65 °C) and the α-skeletal actin gene using *Xba*I or *Bgl*I (blot washing at 72 °C). The $MLC1_A$ ($MLC1_{emb}$) gene was analysed using *Bam*HI as illustrated in Fig. 1.



Fig. 1 *a*, Outline of the backcross strategy between inbred strains of *M. m. domesticus* (DBA/2) and *M. spretus* (SPE/Pas). 42 offspring were analysed for the segregation of RFLPs and 37 for the segregation of biochemical markers. The F_1 males are sterile[22] because of a limited number of loci (J.-L.G., unpublished results). *b*, An example of the segregation of the RFLPs for the $MLC1_A$ ($MLC1_{emb}$) gene. DNA was extracted from the spleen of the parental animals and of the offspring, restricted and analysed on blots as described in ref. 23; 5 μg of each DNA sample were restricted with *Bam*HI and blotted onto nitrocellulose. Blots were probed with the $MLC1_A$ ($MLC1_{emb}$) cDNA labelled by nick-translation as described in ref. 23, then washed in 0.1×SSC at 65 °C before autoradiography. Lanes DBA/2 and Spretus correspond to the parental DNA samples, lanes 1 to 14 to the first 14 back-crosses analysed.

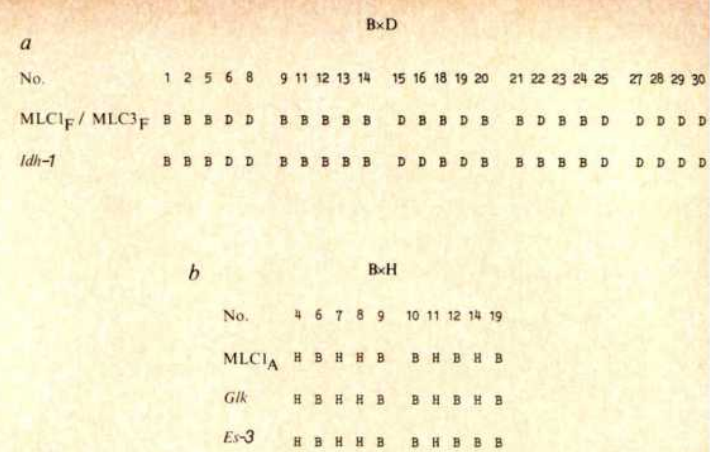


Fig. 2 *a*, Co-segregation of the $MLC1_F/MLC3_F$ and *Idh-1* loci in the B×D recombinant inbred strains. *b*, Co-segregation of the $MLC1_A$ ($MLC1_{emb}$), *Glk* and *Es-3* loci.
Methods. *a*, A *Pst*I RFLP was defined for the myosin light-chain locus between the parental strains. DNA samples of the 24 recombinant inbred strains were purchased from the Jackson laboratory (Bar Harbor) and analysed as described in Fig. 1 legend. *b*, A *Hin*fI RFLP for the MLC locus was used in the same way to follow the segregation of the gene in the 10 B×H recombinant inbred strains. The segregation pattern of *Idh-1*, *Glk* and *Es-3* in these recombinant inbred strains is described in ref. 10.

C57BL/6J (B) and DBA/2J (D) *M. m. domesticus* parental strains[9], in which allelic forms of *Idh-1* segregate[10] (Fig. 2). Of 24 recombinant inbred strains analysed, only two do not show a co-segregation between $MLC1_F/MLC3_F$ and *Idh-1*, indicating a genetic distance of ~2 cM between these loci[9]. Similarly, in B×H recombinant inbred strains derived from C57BL/6J (B) and C3H/HeJ (H)[9], the $MLC1_A$ ($MLC1_{emb}$) locus co-segregates with *Glk* and, with one exception, with *Es-3* (Fig. 2).

The segregation of the genes encoding the α-skeletal and α-cardiac actins[11,12] and a fast skeletal muscle isoform of the myosin heavy chain (MHC_F)[13] was analysed in the same back-cross. Our results confirm that these genes segregate independently, as expected from the analysis of somatic cell hybrids, which allocates them to different mouse chromosomes[14–16]. In addition, we show that they are not linked to myosin light-chain genes in the mouse genome, except for a loose linkage (70% co-segregation) between the MHC_F and $MLC1_A$ ($MLC1_{emb}$) loci, which are both allocated to chromosome 11 (Tables 1, 2). This linkage suggests that the MHC_F gene lies in the middle of chromosome 11 and that the genes are arranged in the order *Hba*/MHC_F/$MLC1_A$/*Es-3* from the centromere on this chromosome.

We conclude, therefore, that the co-expression of contractile protein genes in an adult or fetal, skeletal or cardiac muscle[17] does not require a structural proximity of these genes. Furthermore, the genes coding for different alkali myosin light-chain isoforms, which have probably arisen by gene duplication, have been dispersed in the mouse genome, in contrast to some other multigene families such as that of the β-globin genes[18].

This represents the first report of gene mapping based on the analysis of RFLP segregation in crosses between different mouse species. It is important to show that the evolutionary distance between them does not introduce a bias. For the following reasons, we argue that this is not the case. First, no gross reorganization of the chromosomes has been observed between these species by cytological methods (H. Winking, personal communication). The fact that they breed and give rise to viable and fertile offspring supports this observation. Second, the genes linked on the mouse genetic map co-segregate in our crosses and those which are not linked do not (for example, there are only 8/37 recombinants between *Amy-1* and *Adh-1* (chromosome 3) and 8/37 between *Pgm-1* and *Alb-1* (chromosome 5); see also ref. 19). In addition, our localizations for the $MLC1_F/MLC3_F$ and $MLC1_A$ ($MLC1_{emb}$) genes have been confirmed using recombinant inbred strains.

The type of analysis presented here has several advantages over the use of recombinant inbred strains: (1) The use of

Table 2 Analysis of the segregation of alleles in the back-cross

	$MLC1_A$	$MLC1_V$	α-Skeletal actin	α-Cardiac actin	MHC_F	*Idh*-1	*Es-3*	*Es-14*	*Hba*
$MLC1_F/3_F$	20/42	19/42	22/42	24/42	23/42	35/37	15/37	18/37	18/31
$MLC1_A$		15/42	26/42	26/42	31/42	16/37	34/37	13/37	12/31
$MLC1_V$			21/42	17/42	18/42	18/37	12/37	37/37	17/31
α-Skeletal actin				26/42	21/42	18/37	22/37	18/37	14/31
α-Cardiac actin					25/42	19/37	23/37	16/37	16/31
MHC_F						21/37	27/37	14/37	21/31
Idh-1							17/37	18/37	14/28
Es-3								12/37	11/28
Es-14									16/28

Numbers refer to the numbers of co-segregants per total number of animals analysed.

back-crosses extends the analysis over longer distances (30 cM), whereas recombinant inbred strains tend to keep linked only those loci that are very close together[9]. (2) During the generation of recombinant inbred strains, certain associations of genes may be favoured or selected against, resulting in a nonrandom segregation of loci not observed in a back-cross. (3) The use of distantly related species increases the probability of finding an RFLP for a given gene, so that any gene for which a complementary DNA (cDNA) is available can be analysed.

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1. Solomon, E. & Goodfellow, P. *Nature* **306**, 223-224 (1983).
2. Bonhomme, F. *et al. Biochem. Genet.* **22**, 275-303 (1984).
3. Britton-Davidian, J. & Thaler, L. *Biochem. Genet.* **16**, 213-225 (1978).
4. Bonhomme, F., Martin, S. & Thaler, L. *Experientia* **34**, 1140-1141 (1978).
5. Robert, B. *et al. Cell* **29**, 129-140 (1984).
6. Barton, P. J. R. *et al. J. biol. Chem.* (submitted).
7. Whalen, R. G. & Sell, S. *Nature* **286**, 731-733 (1980).
8. Barton, P. J. R., Robert, B., Fiszman, M. Y., Leader, D. P. & Buckingham, M. E. *J. Muscle Res. Cell Motility* (in the press).
9. Taylor, B. A. in *Origins of Inbred Mice* (ed. Morse, H.) 423-438 (Academic, New York, 1978).
10. *Genetic Variants and Strains of the Laboratory Mouse* (ed. Green, M.)(Fischer, Stuttgart, 1981).
11. Minty, A. J. *et al. J. biol. Chem.* **256**, 1008-1014 (1981).
12. Minty, A. J., Alonso, S., Caravatti, M. & Buckingham, M. E. *Cell* **30**, 185-192 (1982).
13. Weydert, A. *et al. J. biol. Chem.* **258**, 13867-13874 (1983).
14. Czosnek, H. *et al. EMBO J.* **1**, 1299-1305 (1982).
15. Czosnek, H. *et al. EMBO J.* **2**, 1977-1979 (1983).
16. Leinwand, L. A., Fournier, R. E. K., Nadal-Ginard, B. & Shows, T. *Science* **221**, 766-769 (1983).
17. Buckingham, M. in *Essays in Biochemistry* Vol. 20 (eds Campbell, P. N. & Marshall, R. D.)(Academic, New York, 1984).
18. Maniatis, T., Fritsch, E. F., Lauer, J. & Lawn, R. M. *A. Rev. Genet.* **14**, 145-178 (1980).
19. Bonhomme, F., Benmehdi, F., Britton-Davidian, J. & Martin, S. *C.r. hebd. Séanc. Acad. Sci., Paris* **289D**, 545-548 (1979).
20. Harris, H. & Hopkinson, D. A. *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1978).
21. Rougeon, F. & Mach, B. *Gene* **1**, 229-239 (1977).
22. Bonhomme, F., Guénet, J-L. & Catalan, J. *C.r. hebd. Séanc. Acad. Sci., Paris* **294**, 691-693 (1982).
23. Minty, A. J., Alonso, S., Guénet, J-L. & Buckingham, M. E. *J. molec. Biol.* **167**, 77-101 (1983).

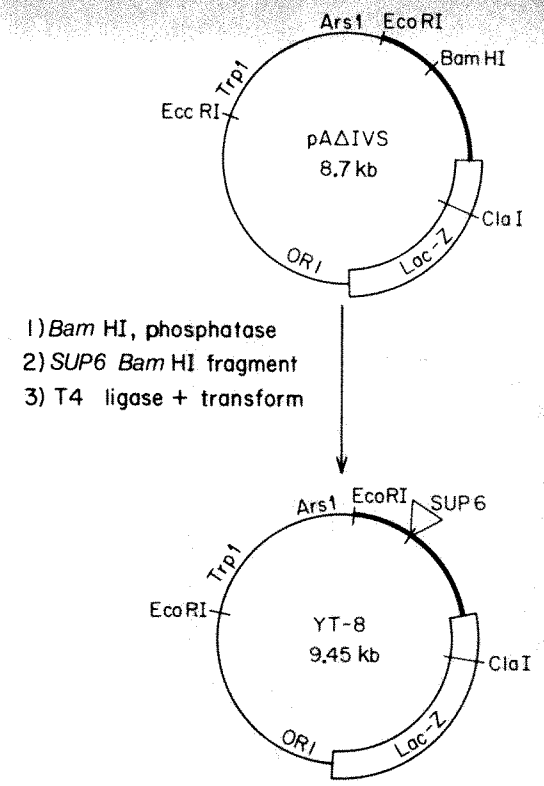


Fig. 1 Construction of plasmid YT-8. Construction of the actin/β-galactosidase fusion plasmid with the actin intron removed (pAΔIVS) has been reported elsewhere[8]. Plasmid YT-8 was constructed by inserting a 0.75-kb *Bam*HI fragment containing the *SUP6* tRNA gene into the *Bam*HI site of pAΔIVS. The thick line indicates the segment containing the yeast actin gene and its flanking sequences.

A test for intron function in the yeast actin gene

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Many eukaryotic genes contain intervening sequences (IVS)[1,2], but the rationale for their existence remains a mystery. Previous studies done in our laboratory demonstrated that the intron in a yeast tRNA^{Tyr} gene, *SUP6*, does have a function[3,4]. We used the same approach to determine the role of introns in nuclear genes encoding messenger RNAs. A single actin gene with one intron exists in *Saccharomyces cerevisiae*[5,6]. The level of actin in yeast appears to be crucial to viability: either too much or too little actin inhibits growth[7]. Therefore, small effects on synthesis of actin protein resulting from the removal of the actin gene intron would be expected to cause measurable changes in cell growth. In the present study, an intron-deleted actin gene was constructed *in vitro* and was used to replace the single resident actin gene in a haploid strain. Analysis of the cells carrying the intron-deleted actin gene shows that the intervening sequence is not essential for actin gene expression.

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Deletion of the intron from a yeast actin/β-galactosidase fusion gene (in pAΔIVS) has been reported previously[8]; this plasmid was modified further by inserting a 750-base pair (bp) *Bam*HI fragment containing the *SUP6* tRNA gene into the unique *Bam*HI site upstream of the actin gene (Fig. 1). *SUP6*, a tyrosine-inserting ochre suppressor, provides a selectable marker for chromosomal integration (Fig. 1) through selection for Leu$^+$ and His$^+$ transformants by *SUP6* suppression of the ochre mutation in those genes. Plasmid DNA from YT-8 (plasmid pAΔIVS with the *SUP6* gene inserted in the *Bam*HI site) was digested with the restriction enzymes *Eco*RI and *Cla*I, then used to transform the haploid yeast strain DBY874 [*α his5-2* (oc), *leu2-1* (oc), *can1-100* (oc), *ura3-52*, *trp5-48* (oc)]. Transformants were initially selected on the basis of their ability to grow on leucine-deficient media. To distinguish from possible Leu$^+$ revertants, the candidates were rescreened for their ability to grow on histidine-deficient media. An alteration of the actin locus could be generated by either genetic recombination (a double crossover between the incoming fragment and the homologous region on the actin gene) or gene conversion (heteroduplex formation between the incoming actin sequences and genomic DNA at the actin locus); either of these mechanisms, or a combination of the two, can be invoked to explain the changes observed in the recombinant actin genes[9].

To determine whether an intron-deleted actin gene had been created by this procedure, genomic DNAs from candidate transformants and from the untransformed parent were compared by Southern blot analysis (Fig. 2). When wild-type yeast DNA is digested with *Eco*RI and *Hin*dIII, two fragments of 1.6 and 2.2 kilobases (kb) are generated which hybridize with a radioactive actin-specific probe[5,6]. As the 1.6-kb fragment contains only the 3′ half of the actin gene, it will remain unchanged in the transformants. The 2.2-kb fragment includes the 5′-flanking

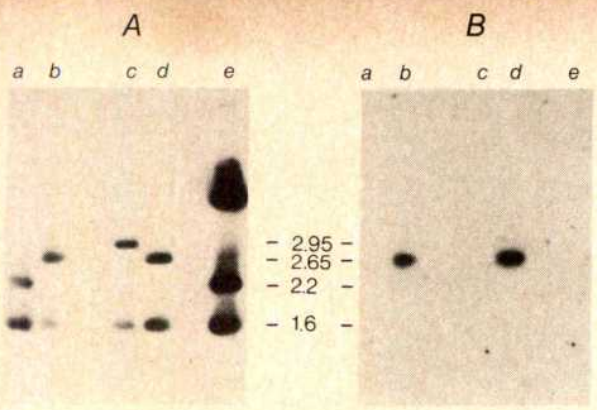


Fig. 2 Southern blot analysis of genomic DNA from DBY874 and transformants 513-A, -V and -L. *A*, Genomic DNA was digested with the restriction enzymes *Eco*RI and *Hin*dIII. After electrophoresis in a 1% agarose gel, DNA was transferred to a nitrocellulose filter and hybridized with nick-translated pYact1 (3.8-kb *Eco*RI fragment containing the actin gene in pBR322; refs 5, 6). All tested strains have the smaller 1.6-kb *Eco*RI/*Hin*dIII fragment in common, whereas the size of the larger *Eco*RI/*Hin*dIII fragment (upper band) differs: *a*, DBY874, 2.2 kb (same size as the wild-type fragment in pYact (*e*); *b*, *d*, 513-A and 513-V, respectively, 2.65 kb (insertion of the *SUP6* gene and deletion of the actin intron); *c*, 513-L, 2.95 kb (insertion of the *SUP6* gene, but with the actin intron present). *B*, The filter in 2*A* was reprobed with a [5'-^{32}P]-labelled 17-base oligonucleotide specific to the actin exon–exon junction.

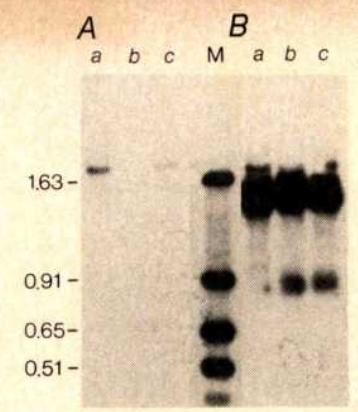


Fig. 3 Northern blot analysis of poly(A)$^+$ RNA from DBY874 and transformants 513-A and 513-L. *A*, Hybridization of poly(A)$^+$ RNA with a nick-translated actin intron-specific fragment (204-bp *Xho*I/*Cla*I fragment of pYact I[5,6]). Actin precursor mRNA (~1,700 nucleotides) is detected only in DBY874 (*a*) and the transformant 513-L (*c*). No actin precursor mRNA is found in strain 513-A (*b*). *B*, The filter used in *A* was rehybridized with nick-translated actin DNA (3.8-kb *Eco*RI fragment of pYact I). Actin mRNA (~1,400 nucleotides) is present in similar amounts in all strains tested. (The probe pYact I also contains a region which hybridized with RNA from the YP2 gene, 800 nucleotides long[11,12].)

Methods. RNAs from DBY874 (*a*), 513-A (*b*) and 513-L (*c*) were isolated from cells grown to mid-logarithmic phase (A_{600} = 2–5) by hot phenol extraction. Poly(A)$^+$ RNA was obtained by oligo(dT)-cellulose chromatography. Glyoxylated poly(A)$^+$ RNA (10 μg) from each strain was electrophoretically separated on a 1.8% agarose gel in 10 mM phosphate buffer[13]. Nucleic acids were transferred to nitrocellulose filters and hybridized according to the procedure of Thomas[14]. Lane M corresponds to 5'-labelled *Alu*I and *Hin*fI fragments of pBR322 serving as size markers.

region and a large portion of the actin gene; the size of this band will vary in transformants because it contains the inserted *SUP6* gene and the actin intron region. Two different classes of transformants were found based on the size of the larger *Hin*dIII/*Eco*RI fragment (Fig. 2*A*): (1) a 2.95-kb band (in 513-L, which has the *SUP6* gene inserted upstream of the actin gene but still contains the actin intron) and (2) a 2.65-kb band (in 513-A and 513-V, which have the *SUP6* gene inserted and no longer contain the intron). No plasmid sequences were present in the transformants (data not shown). Definitive proof of the removal of the actin intron was obtained by hybridization of genomic DNA digested with *Hin*dIII and *Eco*RI with a [5'-^{32}P]-labelled 17-base oligonucleotide specific to the actin exon–exon junction[8]. Only the intron-deleted strains hybridized with the labelled oligonucleotide (Fig. 2*B*).

Expression of the intron-deleted actin gene was analysed by Northern blot analysis of actin-specific mRNA from the wild-type strain and from transformed strains (Fig. 3). Using a nick-translated restriction fragment specific for the actin intron, no actin precursor mRNA could be detected in RNA prepared from the transformant 513-A, whereas it was present in RNA from wild-type strains and from the strain 513-L (Fig. 3*A*). Hybridization of actin-specific DNA, labelled by nick-translation, with poly(A)$^+$ RNA showed that the relative amounts of actin mRNA were roughly equivalent in the strains with and without the actin intron and *SUP6* gene (Fig. 3*B*); however, the level of actin has not been analysed. Further, the strains 513-A and 513-V grew at a rate similar to that of their counterpart 513-L (their doubling times are within 5% of each other).

As the level of actin protein is critical for cell survival, strains 513-A and 513-V must have normal amounts of actin protein[7]. Thus, the deletion of the intervening sequence does not affect the level of actin mRNA in the cell. However, small differences in the level of mRNA and/or actin cannot be ruled out. In addition, there is no apparent effect on sporulation. Diploid cells homozygous for the intron-deleted actin gene sporulate to generate four viable spores (R. Parker, personal communication).

We conclude from this experiment that the intron in the yeast actin gene does not have an observable function. It is possible that the role of the intron is too subtle to be observed in laboratory conditions of growth or that the intron, while having evolutionary significance, has no present role. To conclude that this is true for all yeast genes that contain introns would of course be premature, but there exist strains in which mitochondrial introns have been removed[10] with no observable effect.

Insertion of a selectable marker upstream of the gene of interest may serve as a general method for efficiently transferring mutations into the yeast genome.

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1. Abelson, J. *A. Rev. Biochem.* **48**, 1035–1069 (1979).
2. Breathnach, R. & Chambon, P. *A. Rev. Biochem.* **50**, 349–383 (1981).
3. Wallace, R. B. *et al. Science* **209**, 1396–1400 (1980).
4. Johnson, P. F. & Abelson, J. *Nature* **302**, 681–687 (1983).
5. Gallwitz, D. & Sures, I. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2546–2550 (1980).
6. Ng, R. & Abelson, J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3912–3916 (1980).
7. Shortle, D., Haber, J. & Botstein, D. *Science* **217**, 371–373 (1982).
8. Larson, G. P., Itakura, K., Ito, H. & Rossi, J. *Gene* **22**, 31–39 (1983).
9. Rothstein, R. *Meth. Enzym.* **101**, 202–211 (1983).
10. Jacq, C. *et al. Mitochondrial Genes* (eds Slonimski, P. *et al.*) 155–183 (Cold Spring Harbor Laboratory, New York, 1982).
11. Gallwitz, D., Perrin, F. & Seidel, R. *Nucleic Acids Res.* **9**, 6339–6350 (1981).
12. Gallwitz, D., Donath, C. & Sander, C. *Nature* **306**, 704–707 (1983).
13. McMaster, G. K. & Carmichael, G. G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4835–4838 (1977).
14. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).

Does *Paramecium primaurelia* use a different genetic code in its macronucleus?

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It has long been known that messenger RNAs (mRNAs) of ciliates and in particular of *Paramecium* are not translated well in heterologous *in vitro* translation systems[1–4]. Recently, we have demonstrated for *Paramecium primaurelia* that this phenomenon results from the presence of well-defined blocking sites in the coding sequences of almost all mRNAs, and that these sites are an intrinsic feature of the primary as opposed to the secondary structure of the mRNAs[5]. Here we show that both the gene and the mRNA for the G surface antigen of *P. primaurelia* contain numerous TAA and TAG codons scattered throughout their coding sequences. We propose that these codons do not represent termination codons in *P. primaurelia* but instead code for glutamic acid or glutamine and that the *in vitro* translation of *Paramecium* mRNAs is blocked by their presence.

The G surface antigen is one of the set of surface antigens expressed by *P. primaurelia* in a mutually exclusive manner[6,7]. It is a highly abundant protein covering the whole surface of the cell, and comprises a unique polypeptide chain of relative molecular mass 250,000–300,000 (refs 8, 9). Its mRNA is ~8 kilobases (kb) long and can be detected readily on an RNA gel[4,5,10]. From a genomic library of macronuclear DNA, two *Eco*RI fragments of 5.6 and 1.2 kb, EG1 and EG2, respectively, were selected[5]. Direct proof that the EG1 and EG2 fragments contain sequences coding for the G surface antigen was obtained by selectively hybridizing them with G-antigen mRNA[5,10] and by the direct expression in *Escherichia coli* of subfragments of EG1 and EG2 sequences (E.M., F.C. and A. Baroin, in preparation, and see below for EG2): at least two subfragments, shown in Fig. 1, code for antigenic determinants of the G antigen.

Southern blotting of genomic DNA shows that the G-antigen gene is situated between a *Bam*HI site and the end of a macronuclear chromosome 15.5-kb away; this region is unique in the genome (its restriction map is given in Fig. 1). The gene is oriented with its 3′ end towards the end of the chromosome. By Northern blotting of total RNA from G-expressing paramecia, EG2 is shown to be the most downstream *Eco*RI fragment of the macronuclear chromosome hybridizing to the mRNA of the G antigen (not shown). The precise location of the 3′ end of the transcript was determined by S_1 mapping (not shown) and it is found to lie 70 bp downstream from the *Eco*RV site of EG2 (see Fig. 1). Upstream from this point, the EG2 sequence is completely protected by the mRNA in S_1 protection experiments (not shown).

The entire EG2 fragment was sequenced by the Maxam–Gilbert technique[11]. The strategy used for sequencing is shown in Fig. 1, as is the noncoding strand. This sequence is remarkable in that two regions can be distinguished clearly on the basis of their A+T content (see Fig. 1): the A region at the 5′ end (the first 1,137 base pairs, bp) contains 62% A+T with a marked 3-bp periodicity in their distribution (see Fig. 2*b*) whereas the last 105 bp at the 3′ end (the B region) yields the significantly higher value of 82% A+T and no such periodicity. In organisms having A+T-rich genomes, coding regions usually have a much lower A+T content than noncoding regions and most

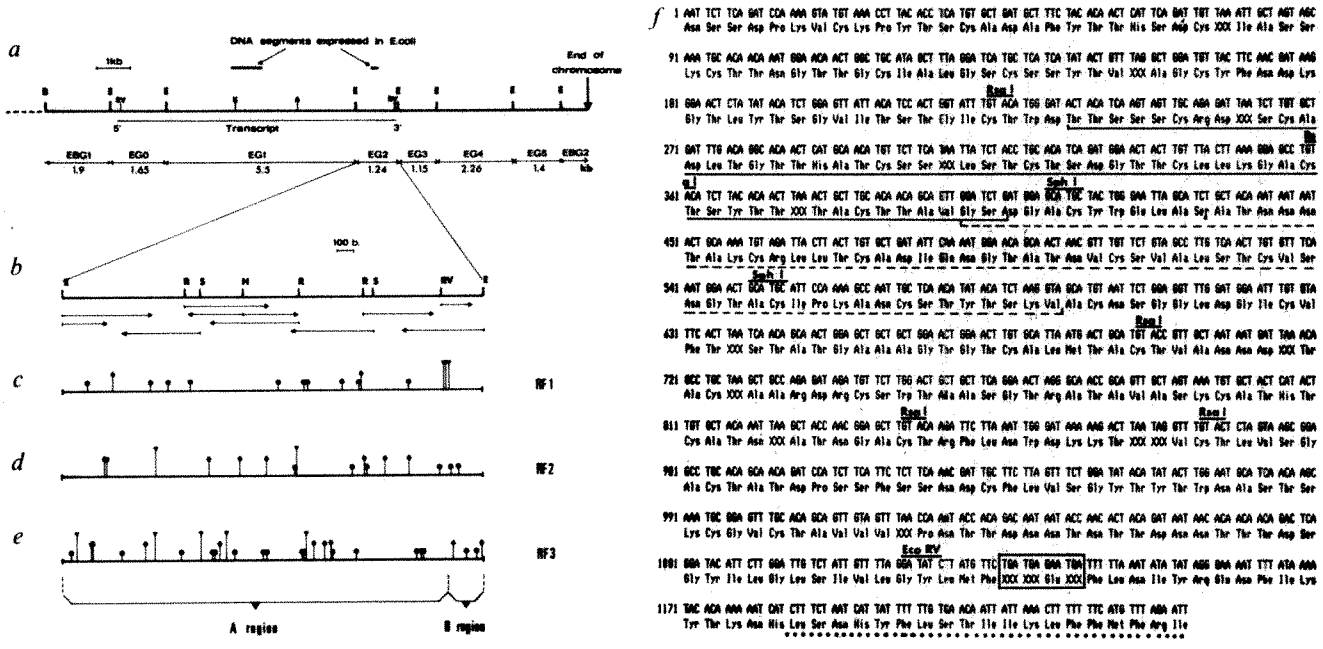


Fig. 1 Structure of the G surface antigen gene and sequence of its 3′ end. *a*, Map of the region of the macronuclear chromosome containing the G surface antigen gene (E, *Eco*RI; B, *Bam*HI; RV, *Eco*RV; A, *Ava*I; K, *Kpn*I; H, *Hin*dII; S, *Sau*3A). After addition of a synthetic *Bam*HI linker to the end of the macronuclear chromosome, we were able to clone the 15.5-kb *Bam*HI fragment in λ EMBL3 (ref. 24). The different *Eco*RI fragments of the cloned 15.5-kb fragment, named (from left to right) EBG1, EG0, EG1, EG2, EG3, EG4, EG5 and EBG2, were subcloned separately in plasmid pUC12 or pBR328. The position of the transcript was determined by S_1 mapping according to ref. 25: the 5′ end is localized in the EG0 fragment, and the 3′ end in the EG2 fragment. The EG2 fragment, which contains the 3′ end of the message, was sequenced (*f*) by the Maxam and Gilbert procedure[11]; the sequencing strategy is shown in *b*. *c–e*, The positions of the stop codons in the three possible reading frames: ●, TAA; ◆, TAG; and ▼, TGA. On the basis of % A+T content, two regions can be distinguished: the A region with 62% A+T and the B region with 82% A+T. *f*, The EG2 sequence. The 1,242-base DNA strand shown is the noncoding strand and the reading frame is RF1. The dotted line indicates the part of the EG2 sequence that is not protected by the mRNA; the dashed line indicates the DNA subfragment containing an antigenic determinant of the G surface antigen (see Fig. 3); solid line, the RNA sequence determined from the *Sph*I/*Sph*I fragment by the primer extension method (see Fig. 4). A cluster of three TGAs near the 3′ end (boxed) is located at the junction between the A and B regions.

a

	RF1	RF2	RF3		RF1	RF2	RF3		RF1	RF2	RF3		RF1	RF2	RF3
TTT	0	10	6	TCT	16	4	4	TAT	6	4	5	TGT	22	3	6
TTC	7	5	13	TCC	1	0	5	TAC	6	2	25	TGC	14	0	22
TTA	9	5	12	TCA	18	2	4	TAA	10	3	15	TGA	0	2	5
TTG	4	7	20	TCG	0	0	0	TAG	2	9	7	TGG	5	1	17
CTT	4	22	5	CCT	1	6	0	CAT	3	24	3	CGT	0	0	2
CTC	0	5	5	CCC	0	0	0	CAC	0	0	14	CGC	0	0	1
CTA	2	15	3	CCA	4	6	0	CAA	1	29	7	CGA	0	0	2
CTG	0	25	6	CCG	0	2	0	CAG	0	19	2	CGG	0	0	3
ATT	8	11	8	ACT	29	3	2	AAT	18	9	3	AGT	4	0	9
ATC	0	3	15	ACC	7	0	2	AAC	6	2	27	AGC	3	0	18
ATA	1	20	9	ACA	34	7	0	AAA	9	5	12	AGA	5	1	9
ATG	2	10	15	ACG	0	4	0	AAG	3	5	4	AGG	1	4	7
GTT	13	8	1	GCT	21	6	0	GAT	17	10	0	GGT	2	0	2
GTC	0	0	2	GCC	6	0	0	GAC	2	0	4	GGC	2	0	2
GTA	6	12	0	GCA	16	11	0	GAA	1	9	1	GGA	24	2	4
GTG	0	8	0	GCG	0	2	0	GAG	0	10	0	GGG	0	4	1

b

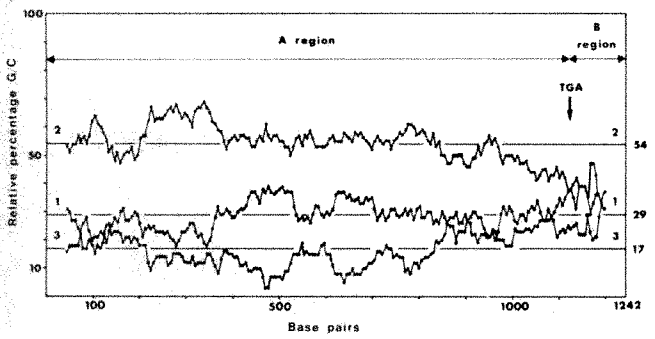


Fig. 2 Codon usage and frequency of G/C bases in the EG2 sequence. *a*, The codon usage in the A region is given for the three possible reading frames RF1, RF2 and RF3. Only RF1 displays a strong bias in favour of A or T at the third position. *b*, The frequency of G/C bases at the three triplet positions. Each point represents the mean value calculated for a 30-triplet fragment (curves 1, 2 and 3 give the relative percentages of G/C at the first, second and third positions, respectively, of the codons in RF1). The three lines are distinct in the A region of the sequence and tend to overlap in the B region. RF1 has the lowest percentage of G/C at the third position.

importantly are characterized by a 3-bp periodicity in A+T richness. As EG2 contains the 3′ end of the mRNA, the existence of these two very distinct regions suggests that region A could be the end of the coding sequence. However, the three possible reading frames RF1, RF2 and RF3 all contain scattered TAA, TAG and TGA codons, found every 80 bp on average (see Fig. 1). Yet, if one looks more carefully at the other codons used in the short open reading frames (in between two stop codons) in the A region, one finds that RF1, but not the other two reading frames, shows the following characteristics: (1) The 3-bp periodicity mentioned above falls so that the third base of each codon shows the strongest bias for A and T (see Fig. 2), characteristic of coding sequences in A+T-rich genomes. (2) The ratio of RNY to YNR codons, where R is a purine, N any base and Y a pyrimidine, is equal to 2.5 for the reading frame FR1 and 0.4 and 0.9 for RF2 and RF3 (see Table 1). It has been observed[12,13] that in many prokaryotic and eukaryotic genomes this ratio is >2 for coding frames and <1 for noncoding frames. (3) The amino-acid composition deduced from RF1 is remarkably close to that of the entire G surface antigen (see Table 1). χ^2 values calculated from the amino-acid composition of G surface antigen[14] are 47 for RF1, 740 for RF2 and 650 for RF3. The same calculation for the last 110 bp of the EG2 sequence (region b), whatever the reading frame, yields higher χ^2 values than for any 110-bp stretch of the A region in RF1 (see Table 1, column *i*, in which a comparison is made with the first 110 bp of the A region). (4) The nature of the stop codons in RF1 is also peculiar as only TAA and TAG codons are present in region A and a cluster of three TGA codons is located at the junction between A and B (see Fig. 1).

The above arguments, especially those concerning the RNY/YNR ratio, the 3-bp periodicity and the χ^2 values, strongly support the notion that, despite the presence of TAA and TAG triplets in RF1, the A region codes for part of the G antigen.

To obtain direct proof that the A region contains coding sequences and that RF1 is the proper reading frame, we used direct expression of DNA subfragments of EG2 in the expression vector λ gt11 to determine whether they code for antigenic determinants which can be recognized by antibodies against G surface antigen. EG2 was cleaved with the restriction enzyme *Rsa*I (see Fig. 1), and digested with exonuclease *Bal*31 to generate DNA subfragments of various lengths, which were then

Table 1 Amino-acid compositions of A and B regions of EG2 sequence

	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*
Phe	5.8	7	13	19	0.6	6	5	7	1
Leu	17.9	19	79	51	1.8	4	5	1	0
Ser	26.3	42	6	38	2.7	2	1	3	6
Tyr	11.9	12	6	30	1.2	3	1	3	2
Cys	39.5	36	3	28	4.0	0	2	0	4
Trp	4.1	5	1	17	0.4	0	0	0	0
Pro	7.2	5	14	0	0.7	0	0	0	2
His	3.3	3	24	17	0.3	2	0	2	1
Glx	21.5	2	67	10	2.2	2	5	0	0
Arg	5.4	6	5	24	0.5	2	0	1	0
Ile	9.0	9	34	32	0.9	5	8	3	1
Met	1.9	2	10	15	0.2	1	0	1	0
Thr	57.5	70	14	4	5.8	2	1	1	7
Asx	42.2	43	21	34	4.3	4	5	3	5
Lys	21.5	12	10	16	2.2	3	2	5	3
Val	16.4	19	28	3	1.7	0	0	2	1
Ala	47.7	43	19	0	4.8	0	0	0	3
Gly	36.0	28	6	9	3.6	0	1	1	1
TAA		10	3	15		0	2	2	1
TAG		2	9	7		0	0	2	0
TGA		0	2	5		3	0	0	0
Total	375	375	375	375	39	39	39	39	39
χ^2		38	709	583		107	220	112	17
RNY/YNR		2.5	0.4	0.9		0.9	0.8	1.5	2.6

Amino-acid compositions deduced for the A region (columns *b*, *c*, *d*) and the B region (columns *f*, *g*, *h*) of the EG2 sequence in the three reading frames RF1 (*b*, *f*), RF2 (*c*, *g*) and RF3 (*d*, *h*) and for the first 110 bp of the EG2 sequence in RF1 (column *i*). The amino-acid composition of the G antigen[15] is given for comparison in columns *a* and *e*. χ^2 values and the RNY/YNR ratio are also given.

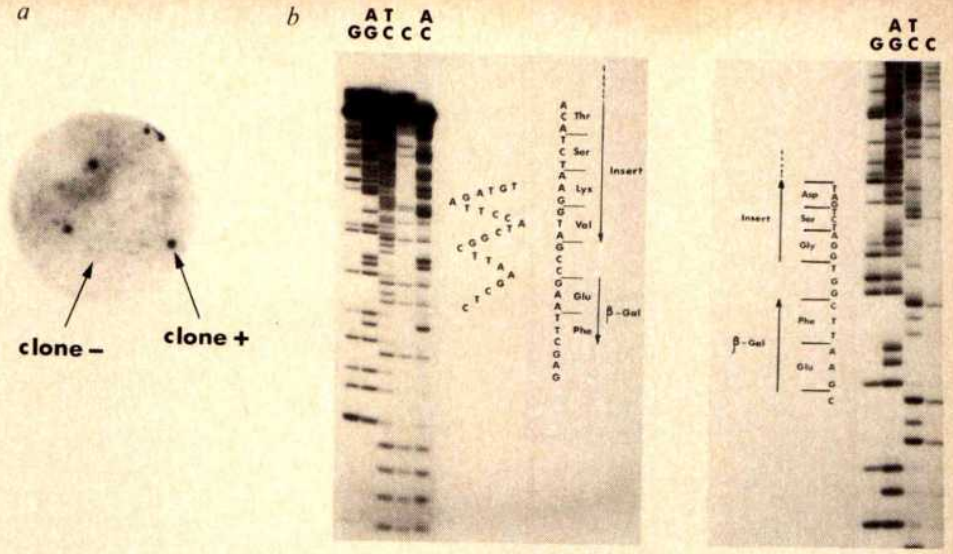


Fig. 3 Determination of the reading frame of the coding sequence of the G surface antigen. 50 μg of plasmid pEG2 (recombinant pBR328 plasmid containing the EG2 sequence at the *Eco*RI site) were digested to completion with the restriction enzyme *Rsa*I and then with 1 unit of *Bal*31 nuclease (Boehringer) at 37 °C. Aliquots of the reaction mixture were removed after 1, 2, 3, 4, 5, 6, 8 and 10 min of digestion, phenol-extracted and pooled for ethanol precipitation. After repair of the fragments with *E. coli* DNA polymerase, synthetic ^{32}P-labelled *Eco*RI linkers (P.L. Biochemicals) were ligated to their ends. After digestion with excess *Eco*RI, the DNA fragments (separated from the *Eco*RI linkers by two successive G50 column chromatography steps) were used to produce an expression library in the λ gt11 vector[15] at the *Eco*RI site. The library was screened with antisera-purified antibodies against purified G surface antigen, depleted of any anti-*E. coli* activities. Many positive recombinants were obtained and subcloned twice. ***a***, Positive (+) and negative (−) clones from a nitrocellulose replica of phage plaques after incubation with these antibodies, followed by labelling with ^{125}I-protein A (Amersham). The insert of one of the positive clones was subcloned in the two possible orientations in the pUC12 vector and its sequence determined by the Maxam and Gilbert technique[11] from both ends; it corresponds exactly to the sequence indicated by a dashed line in Fig. 1. Special care was taken to establish the sequence around the two *Eco*RI junctions as it gives the reading frame of the coding sequence of the insert (see text): the two recombinant plasmids bearing the insert in either of the two possible orientations were labelled at the *Bam*HI site, which is contained in the pUC12 polylinker 18 bases from the *Eco*RI site, and were sequenced from the *Bam*HI site towards the *Eco*RI site by the Maxam–Gilbert technique[11]. The two 20% acrylamide sequencing gels in ***b*** show that the reading frame of the insert is RF1, as deduced from that of the β-galactosidase.

inserted into the λ gt11 β-galactosidase expression vector[15]. Recombinants synthesize hybrid proteins made up of β-galactosidase and part of the polypeptide chain of the G surface antigen, provided the subfragments encode part of the G antigen and are inserted in the correct orientation and reading frame. Furthermore, if the expressed part of the G surface antigen contains an antigenic determinant, the clones synthesizing hybrid proteins can be detected directly by using antibodies against G antigen.

We obtained recombinant clones synthesizing polypeptides that reacted with antibodies against G antigen; one of these clones has been analysed in detail (see Fig. 3). The sequence of the insert is indicated by a dashed line in Fig. 1; this sequence is unique in EG2 and in the entire 15.5-kb fragment. By sequencing the junctions around the *Eco*RI sites, the reading frame of the insert can be deduced from that of β-galactosidase. From the sequences shown in Fig. 3, we conclude that RF1 is the correct reading frame of the G antigen.

Although it is clear that the sequence marked by a dashed line in Fig. 1 contains an antigenic determinant of the G surface antigen and that the open reading frames surrounding this fragment are probably part of the coding sequence as shown above, the punctuation of the coding sequence with TAA and TAG stop codons remains puzzling. One possible explanation is that they are removed by splicing. In order to demonstrate that this is not the case, we sequenced the cytoplasmic mRNA by the primer extension method[16], using as primer the *Sph*I/*Sph*I fragment included in the fragment expressed in *E. coli* (see Fig. 1). The sequence of the mRNA (Fig. 4) corresponds exactly to the sequence underlined with a solid line in Fig. 1. We conclude from these data that TAA and TAG codons are present in the reading frame of the coding sequence of the G-antigen mRNA.

Thus, TAA and TAG do not represent termination codons in *P. primaurelia*. The simplest explanation of the results is that they code for amino acids; other possible interpretations seem highly unlikely. For example, it could be argued that the gene we have cloned is a pseudogene[17] and that the true gene does not contain TAA and TAG triplets. This interpretation is not plausible for the following reasons: (1) Our S_1 mapping (not shown) and RNA sequencing experiments indicate that the cloned gene is transcribed abundantly. (2) If the cloned gene is a pseudogene, it must share large segments of homology with the true gene[17]. S_1 protection experiments (not shown) performed with EG1, EG2 or subfragments of these have never yielded anything other than a single protected fragment, indicating that the cloned gene and the true gene would differ at most by local changes not revealed by S_1 protection experiments. The 195-bp *Sph*I/*Sph*I primer, which does not contain TAA and TAG codons and which is, moreover, included in a 336-bp fragment coding for an antigenic determinant of the protein, should also hybridize with the mRNA of the true gene. As the sequence of the cloned gene alone is read and as it is read without ambiguity (see Fig. 4), the true gene should be transcribed at a much lower rate than the cloned gene (that is, at an undetectable level), which is highly unlikely for such an abundant protein. (3) Southern blots of total DNA of *Paramecium* and of the 15.5-kb cloned fragment cut with various restriction enzymes and probed with EG1 or EG2 fragments have always been found to be identical (not shown); this is at variance with the frequent observation that pseudogenes and true genes give rise to different sets of bands on Southern blots[17]. (4) The 10 TAA and 2 TAG codons in the EG2 sequence do not seem to be generated by the random mutations expected in a pseudogene. Indeed, despite their presence, the reading frame is unchanged over the whole length of the coding sequence.

Our results lead us to the following conclusions: (1) The fact that TAA and TAG codons are interspersed in the coding sequence (see accompanying paper[18]) may explain the blocking of *in vitro* translation of *Paramecium* mRNAs. (2) The localization of the three TGAs in a cluster of four codons just before the end of the transcript, and at the junction between the A and B regions of the gene, suggests strongly that TGA is a termination codon in *Paramecium*. Note that the few genes sequenced to date in ciliates[19–21] all use TGA termination codons. (3) If TAA and TAG are coding triplets in *Paramecium*, we propose that they code for glutamic acid or glutamine on the basis of their important contributions (30%) to the χ^2 value. We do not think that many other changes in the *Paramecium* codon assignment

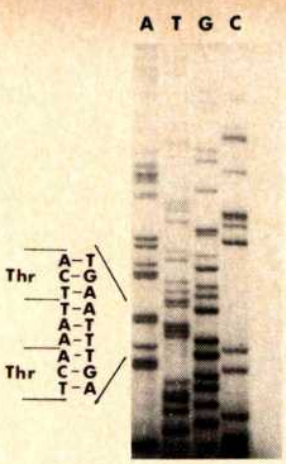


Fig. 4 RNA sequencing gel obtained from the *Sph*I/*Sph*I fragment by the primer extension method. Plasmid pEG2 (15 μg) was cut with *Sph*I and the fragments generated were labelled at their 5′ ends with [γ-^{32}P]ATP using polynucleotide kinase. The 138-bp long *Sph*I/*Sph*I fragment contained in EG2 (see Fig. 1) was purified on a polyacrylamide gel; 25% of the recovered probe and 75 μg of total RNA from G-expressing paramecia were ethanol-precipitated and resuspended in 80 μl of 80% formamide hybridization buffer[16]. The solution was warmed to 80 °C before being transferred quickly to a 60 °C water bath, the temperature of which was allowed to cool down to 30 °C over 2–3 h. Nucleic acids were precipitated with ethanol and polymerization was performed with reverse transcriptase (Life Science) and a mixture of the four deoxy- and dideoxynucleotides[16]. Part of the 6% acrylamide sequencing gel shown gives the RNA sequence around the first TAA codon upstream of the primer.

have occurred as translation of the EG2 sequence according to the universal code leads to a good overall agreement with the amino-acid composition of the G antigen. Direct proof that the TAA and TAG triplets encode amino acids in *Paramecium* has yet to be obtained, and would best comprise a direct comparison of the sequence of a protein with that of its gene, and the demonstration that transfer RNAs bearing anticodons to TAA and TAG triplets exist.

Some evidence that TAA encodes an amino acid in *Tetrahymena*[22] and *Stylonichia*[23] has been reported recently; this deviation from the universal code might be peculiar to all ciliates. It would be interesting to know whether it is linked to the existence of nuclear differentiation in ciliates and in particular whether the micronuclear and macronuclear copies of the genes are identical in this respect. This first example of an altered genetic code apart from that in mitochondria may provide new clues as to the evolution of the genetic code.

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1. Hruby, D. E., Maki, R. A. & Cummings, D. J. *Biochim. biophys. Acta* **477**, 89–96 (1977).
2. David, E. T. & Smith, K. E. *Biochem. J.* **194**, 761–770 (1981).
3. Rinaldy, A. R., Wethoff, P., Janker, F., Seyfert, H. M. & Cleffmann, G. *Expl Cell Res.* **134**, 417–423 (1981).
4. Preer, J. R., Preer, L. B. & Rudman, B. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6776–6778 (1981).
5. Meyer, E., Caron, F. & Guiard, B. *Biochimie* **66**, 403–412 (1984).
6. Beale, G. H. *Genetics of* Paramecium aurelia (Cambridge University Press, 1954).
7. Capdeville, Y. *Molec. gen. genet.* **11**, 306–316 (1971).
8. Hansma, H. G. *J. Protozool.* **22**, 257–259 (1975).
9. Reisner, A. H. & Rowe, J. *Nature* **222**, 558–559 (1969).
10. Forney, J. D. *et al. Molec. cell. Biol.* **3**, 466–474 (1983).
11. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
12. Eigen, M. *Naturwissenschaften* **65**, 341–369 (1978).
13. Schepherd, J. C. W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1596–1600 (1981).
14. Jones, I. G. *Biochem. J.* **96**, 17–23 (1965).
15. Young, R. A. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1194–1198 (1983).
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
17. Little, P. F. R. *Cell* **28**, 683–684 (1982).
18. Preer, J. R. Jr., Preer, L. B., Rudman, B. M. & Barnett, A. J. *Nature* (this issue).
19. Klobutcher, L. A., Jahn, C. L. & Prescott, D. M. *Cell* **36**, 1045–1055 (1984).
20. Bannon, G. A., Bowen, J. K., Yao, M. C. & Gorovsky, M. A. *Nucleic Acids Res.* **12**, 1961–1975 (1984).
21. Kaine, B. P. & Spear, B. B. *Nature* **295**, 430–432 (1982).
22. Horowitz, S. & Gorovsky, M. A. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
23. Helftenbein, E. *Nucleic Acids Res.* (submitted).
24. Frischauf, A. M., Lehrach, H., Poustka, A. M. & Murray, N. *J. molec. Biol.* **170**, 827–842 (1983).
25. Farrell, P. J., Deininger, P. L., Bankier, A. & Barrell, B. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1565–1569 (1983).

Deviation from the universal code shown by the gene for surface protein 51A in *Paramecium*

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The immobilization antigens (i-antigens) of *Paramecium*, large polypeptides of relative molecular mass ~300,000 (ref. 1), are located on the cell surface. Each i-antigen is encoded by a different unlinked gene, and no more than one gene is expressed at a time. The proteins and the mRNAs and genes encoding them are readily isolated[2,3]. Here we report the nucleotide sequence of three regions of the A i-antigen gene from stock 51 of *Paramecium tetraurelia*. Surprisingly, all reading frames contain TAA and TAG stop codons, even though there is evidence that one reading frame of these sequences codes for the i-antigen. We suggest that in *Paramecium* UAA and UAG code for amino acids, instead of serving as translational stops as they do in all other organisms.

λ SA-1 contains the whole transcribed portion of the A gene, ~8.4 kilobases (kb), together with the 5′ and 3′ flanking regions[3]. We sequenced 681 bases at the 5′ end of the gene and ~400 bases farther downstream (Fig. 1). The start of transcription has been localized previously in an S_1 protection experiment to a point ~450 bases to the left of a *Hin*dIII site[3]. In a similar S_1 nuclease experiment, using a shorter fragment and determining more precisely the size of the protected fragment on a sequencing gel (data not shown), the start of transcription was more precisely located 470 bases to the left of the *Hin*dIII site.

Each of the reading frames subsequent to the first ATG (+15) after the start of transcription (+1) has TAA or TAG stop codons

Table 1 Analysis of reading frames

Region of gene	Reading frame	No. of stop codons TAA	TAG	TGA	Fit to protein	RNY:YNR
−181 to +14	1	3	1	3	182	18:18
	2	5	0	3	102	16:20
	3	5	0	3	108	22:15
+15 to +681	1	4	2	0	26	76:34
	2	5	3	3	503	36:65
	3	5	5	12	404	52:64
+1,126 to +1,291	1	1	0	0	13	21:7
	2	2	0	0	205	4:15
	3	5	1	2	113	13:17
+5,486 to +5,692	1	3	1	0	17	30:8
	2	0	0	0	173	18:22
	3	4	1	5	147	6:24

Each of three possible reading frames is analysed for four regions of the 51A genes. The last three regions are believed to be translated. The number of stop codons, χ^2 fit to overall protein composition and ratio of RNY to YNR codons are given (R is purine, Y is pyrimidine and N is any base).



Fig. 1 Restriction map of the *Paramecium* DNA inserted into λ SA-1, and the nucleotide sequence of four regions of the 51A gene. H refers to *Hin*dIII; R is *Eco*RI. The arrow below the restriction map represents the transcribed portion of the gene. Sequences were obtained by using the Maxam-Gilbert technique[15] with minor exceptions both strands were sequenced at least twice.

(Table 1), except for the second reading frame of the 205 bases starting at position +5,485. It is our contention that all these regions (except for the sequences from −181 to +15 or somewhat beyond) are transcribed and their messages translated. For each region the true reading frame is thought to be the first one listed in Table 1, for the following reasons. (1) The presence of introns is unlikely as judged by S_1 nuclease experiments. In addition to the S_1 experiments already cited, comparable experiments (data not shown) have ruled out the possibility of introns in the first 2,900 bases. Indeed, the whole 8.4-kb transcribed region of the gene is just large enough to code for the poly(A)RNA with which it hybridizes[2,3], and either a TAA or a TAG occurs on average once every 100 bases. Moreover, the characteristic sequences at the boundaries of introns are not found.

(2) It is unlikely that the gene sequenced is a pseudogene or a regulatory gene. Correlations between the size and abundance of the i-antigens and a series of poly(A)$^+$ RNAs provide strong evidence that the RNAs code for the i-antigens[2]. The sequences in λ SA-1 hybridize to the RNA judged to be the mRNA for i-antigen A. Genomic Southern transfer experiments indicate that there is probably only one copy of the sequences in the SA-1 genome[3]. Furthermore, there are deletion mutations that eliminate these sequences and simultaneously eliminate the ability of stock 51 to produce i-antigen A[4]. We have crossed a strain of stock 51 bearing a deletion of the gene found in λ SA-1 with another stock of *P. tetraurelia* (stock 29) bearing the A allele. Alleles 29A and 51A determine i-antigens that can be distinguished serologically[1]. Antigen 29A and the deletion were found in the F_2 offspring in a 1:1 ratio, as expected if the deletion included the sequences responsible for serological specificity of the A protein. These results provide strong evidence that the gene found in λ SA-1 codes for the A protein.

Table 2 Amino acid frequencies

	Obs.	Exp.		Obs.	Exp.
Val	19	15	His	3	2
Leu	19	13	Asn + Gln	26	35
Ile	6	8	Ser	28	33
Phe	5	5	Thr	52	55
Ala	37	40	Gly	13	23
Pro	8	6	Cys	41	37
			Tyr	11	9
Trp	6	5			
Met	4	1	TAA	8	
Asp + Asn	46	39	TAG	3	
			TGA	0	
Glu + Gln	12	20			
Lys	20	15			
Arg	4	4			

Comparison of numbers of amino acids predicted from observed (obs.) codons with numbers expected from amino acid analysis of whole protein (exp.).

(3) It is unlikely that the sequences result from cloning artefacts. We have sequenced a small region of an independently isolated clone, λ SA-2, and find that it agrees with the sequence from +360 to +469 of λ SA-1 (Fig. 1), and includes a TAA and a TAG triplet.

(4) One of the three reading frames predicts an amino acid composition very similar to that estimated for immobilization antigen 51A by conventional amino acid analysis[5,6]; the fit was computed as a χ^2 value calculated from the observed composition of the whole protein and the composition of a given fragment deduced from the sequence (omitting TAA and TAG codons). These values are given in Table 1 for each reading frame for each region. As the sequences represent a significant portion of the whole protein and some of the classes of amino acids overlap, the assumption of independence does not hold, and a true χ^2 distribution is not expected. Nevertheless, χ^2 is a useful measure of the fit. In each case the first reading frame in each translated region has a very low χ^2 value and the other two frames predict a composition very far from the known composition of the whole protein.

(5) Codon usage suggests that the first reading frame is the correct one. Codon usage in all organisms is restricted such that the initial base in each codon is frequently a purine (R), the last base a pyrimidine (Y), while the middle base may be any base (N)[7]. The ratio of RNY to YNR codons is almost always ≥2:1 in true reading frames. The RNY/YNR ratios in Table 1 indicate that the first reading frame of each region represents the translated sequence.

To explore possible amino acid assignments for TAA and TAG, the frequencies of amino acids predicted by the observed codons for all three translated regions are given in Table 2, together with the numbers expected from the overall amino acid composition of i-antigen A. Ignoring TAA and TAG, the χ^2 for these observed and expected values is 23.0. If TAA and TAG are assigned to specific amino acids, only three of the 20 possible assignments result in lower χ^2 values: Glu ($\chi^2 = 19.9$), Gly (18.8) and Gln (16.5). Therefore, the data show that the best fit is obtained if glutamine is encoded by TAA and TAG. Normal codons for glutamine are CAA and CAG, both of which are found in our sequences. The data of Caron and Meyer[8] show that the level of Glu + Gln is low. Helftenbein[9] reports a TAA that appears to specify glutamine in several α-tubulin genes in *Stylonychia*, and Horowitz and Gorovsky[10] have found a TAA specifying glutamine in a histone gene in *Tetrahymena*. Perhaps TAA specifies glutamine in all ciliated protozoa. Finally, the total of 345 codons in reading frame 1 includes 8 TAA, 3 TAG and 0 TGA, suggesting that UGA alone is used as a translational stop by *Paramecium*.

The use of UAA and UAG codons by ciliates should make their mRNAs poor templates for *in vitro* protein synthesis in heterologous systems, indeed numerous workers have found this to be the case [2,11-13]. Meyer *et al.* have concluded recently that the primary structure of the mRNAs from *Paramecium* is at fault[14].

We conclude that UAA and UAG do not represent stop codons in *Paramecium*; the only other deviations from the 'universal' genetic code have been found in mitochondria.

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1. Sonneborn, T. M. in *Handbook of Genetics* Vol. 2 (ed. King, R. C.) 469-594 (Plenum, New York, 1975).
2. Preer, J. R., Preer, L. B. & Rudman, B. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6776-6778 (1981).
3. Forney, J. D. *et al. Molec. cell. Biol.* **3**, 466-474 (1983).
4. Epstein, L. M. & Forney, J. D. *Molec. cell. Biol.* **4**, 1583-1590 (1984).
4. Steers, E. Jr *Biochemistry* **4**, 1896-1901 (1965).
6. Reisner, A. H. & Rowe, J. *Nature* **222**, 558-559 (1969).
7. Shepherd, J. C. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1194-1198 (1981).
8. Caron, F. & Meyer, E. *Nature* **314**, 185-188 (1985).
9. Helftenbein, E. *Nucleic Acids Res.* (submitted).
10. Horowitz, S. & Gorovsky, M. *Proc. natn. Acad. Sci. U.S.A.* (submitted).
11. Hruby, D. E., Maki, R. A. & Cummings, D. J. *Biochim. biophys. Acta* **477**, 89-96 (1977).
12. David, E. T. & Smith, K. E. *Biochem. J.* **194**, 761-770 (1981).
13. Rinaldy, A. R., Wethoff, P., Janker, F., Seyfert, H. M. & Cleffmann, G. *Expl Cell Res.* **134**, 417-423 (1981).
14. Meyer, E., Caron, F. & Guiard, B. *Biochimie* **66**, 403-412 (1984).
15. Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560-564 (1977).

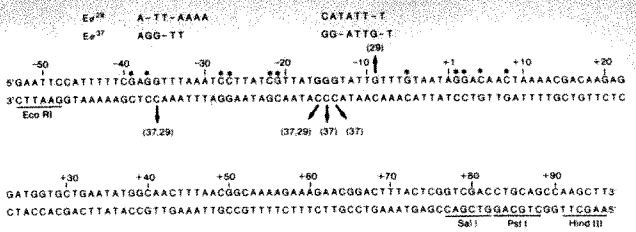


Fig. 1 Structure of the *ctc* promoter region, showing the nucleotide sequence of the *ctc* promoter region which was cloned into M13 mp9 and then recloned into pUC8. Transcription proceeds from left to right, with the lower strand representing the transcribed strand. The start point of transcription is indicated as nucleotide +1. Asterisks represent the positions at which base substitutions (transitions) have little effect on the utilization of this promoter by $E\sigma^{37}$ (ref. 6) or $E\sigma^{29}$. The arrows indicate those positions at which single base transitions more dramatically affect the utilization of the promoter by $E\sigma^{37}$ or $E\sigma^{29}$ as indicated (arrows pointing down indicate a decrease in utilization and the arrow pointing up indicates an increase in utilization). The nucleotide sequences shown above the *ctc* promoter sequence are the sequences (non-transcribed strand shown) which are conserved in promoters recognized by $E\sigma^{37}$ or $E\sigma^{29}$ as indicated[6,8].

Utilization of one promoter by two forms of RNA polymerase from *Bacillus subtilis*

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***Bacillus subtilis* possesses several forms of RNA polymerase, each differing in its σ subunit and its specificity of promoter recognition[1,2]. The sequential appearance of σ subunits, which change the promoter recognition specificity of RNA polymerase, may have a key role in controlling the temporal pattern of gene expression required for endospore development in *B. subtilis*[3]. Several genes that are expressed over relatively long periods of time during the developmental cycle are transcribed by more than one form of RNA polymerase, which initiate transcription from either tandem or overlapping promoters[2,4]. The promoter region for the *ctc* gene is interesting because transcription is initiated at or near the same position by both σ^{37} RNA polymerase ($E\sigma^{37}$), a minor form in growing cells, and σ^{29} RNA polymerase ($E\sigma^{29}$), a form which appears ~2 h after the initiation of sproulation[5]. Here we report that several base substitutions in the *ctc* promoter differentially affect the utilization of the promoter by $E\sigma^{37}$ or $E\sigma^{29}$.**

We have reported previously the use of site-directed bisulphite mutagenesis to construct a series of single and multiple base substitutions in the *ctc* promoter[6]. For this purpose, we cloned a 130-base pair (bp) DNA fragment containing the *ctc* promoter into the *Escherichia coli* phage M13 mp9 to produce phage M13-31 (Fig. 1). After mutagenesis and transfection[6], the 143-bp *Eco*RI/*Hin*dIII DNA fragment containing the promoter region was excised from each phage DNA and ligated between the *Eco*RI and *Hin*dIII restriction sites of plasmid pUC8 (ref. 7). The structure of each plasmid was confirmed by determining the nucleotide sequence of the *Eco*RI/*Hin*dIII DNA fragment (Table 1).

To test the function of the mutant promoters, we assayed their ability to direct transcription by $E\sigma^{29}$ and $E\sigma^{37}$ in an *in vitro* run-off assay. Template DNA was prepared by cutting the plasmid DNA at the unique *Hin*dIII restriction site 95 bp downstream from the startpoint of transcription (Fig. 1). $E\sigma^{37}$ or $E\sigma^{29}$ RNA polymerase was incubated with an excess of the cut template DNA and the discrete run-off transcripts visualized by autoradiography after electrophoresis in a polyacrylamide/urea gel. Figure 2 shows the transcripts generated from a series of mutant promoters with single base substitutions at positions occupied by guanine in the non-transcribed stand of the wild-type promoter. As we had shown previously using phage replicative-form DNA as template[6], the single base pair substitutions causing the greatest reduction in promoter utilization by $E\sigma^{37}$ were at positions −36, −16, −15 and −14 (Fig. 2, lanes *d*, *f*, *g*, *h*, respectively, and Table 1).

Each of the cut templates was also transcribed by an equal amount of $E\sigma^{29}$ in reactions that otherwise were identical to the $E\sigma^{37}$ transcriptions. Transcription of the wild-type template by $E\sigma^{29}$ results in a 95-base transcript which co-migrates during electrophoresis in a high-resolution nucleotide sequencing gel with the 95-base transcript generated by $E\sigma^{37}$ (K.M.T. and C.P.M. Jr, unpublished data). We also observed some non-specific transcription products that migrate more slowly than the 95-base run-off transcript (Fig. 2, lane *k*). The intensity of this nonspecific background did not correlate with the intensity of the run-off transcript (Fig. 3, compare lanes *f* and *g*).

Transcription of this series of cut templates by $E\sigma^{29}$ revealed that the base substitution at position −15, which greatly reduced transcription by $E\sigma^{37}$, had little effect on transcription by $E\sigma^{29}$ (Fig. 2, lane *q*). This template was an aliquot of the same cut template used for transcription by $E\sigma^{37}$ (Fig. 2, lane *g*), thereby providing additional evidence that the inefficiency of transcription of this template by $E\sigma^{37}$ was not caused by the quality of the template preparation (also see mixing experiments in ref. 6). The base substitution at position −9 had little or no effect on transcription by $E\sigma^{37}$, but we observed a slight enhancement of transcription from this template by $E\sigma^{29}$ (Fig. 2, lane *s*).

Promoters containing single base substitutions at positions −36 or −16 directed less transcription by $E\sigma^{29}$ (Fig. 2, lanes *n*, *p*; Table 1). Mixing experiments were used to eliminate the possibility that these template preparations contain an inhibitor of $E\sigma^{29}$. When *Pst*I-cut wild-type template was mixed with each *Hin*dIII-cut mutant template in pre-binding transcription reactions with $E\sigma^{29}$, the mutant templates did not inhibit transcription from the wild-type template (data not shown).

We have also tested promoters containing multiple base substitutions in similar pre-binding transcription reactions (Table

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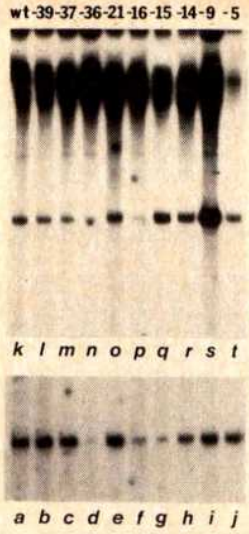


Fig. 2 Run-off transcripts from mutant promoters. $E\sigma^{37}$ from *B. subtilis* JH646 (*spoOA*) was purified as described elsewhere[6] and was shown to contain no contaiminating $E\sigma^{32}$ or $E\sigma^{29}$ by *in vitro* transcription of plasmids pCB1291 and pMB9, which contain promoters utilized uniquely by $E\sigma^{32}$ and $E\sigma^{29}$, respectively[2,5]. The results were reproduced with $E\sigma^{37}$ isolated from *B. subtilis* (SMY). $E\sigma^{29}$ was purified on two different occasions from *B. subtilis* (SMY) by gradient elution from DNA-cellulose as described by Haldenwang *et al.*[5] and shown to be free of contaminating $E\sigma^{32}$ and $E\sigma^{37}$ by transcription of pCB1291, which contains promoters used uniquely by $E\sigma^{37}$ and $E\sigma^{32}$ (ref. 2). We have not excluded the possibility that in addition to their sigma subunits, these holoenzymes differ by modifications that cannot be detected by electrophoresis in SDS-polyacrylamide gels. Addition of a mixture of $E\sigma^{37}$ and $E\sigma^{29}$ to a mutant template has the expected additive effect; therefore, the differential effects are not due to impurities in the polymerase preparations. RNA was transcribed as described previously[6] by $E\sigma^{37}$ (lanes *a–j*) or $E\sigma^{29}$ (lanes *k–t*) from the following templates that had been cleaved at the *Hin*dIII site 95 bp downstream from the *ctc* promoter: pUC8-31, lanes *a, k*; pUC8-39, lanes *b, l*; pUC8-37, lanes *c, m*; pUC8-36, lanes *d, n*; pUC8-21, lanes *e, o*; pUC8-16, lanes *f, p*; pUC8-15, lanes *g, q*; pUC8-14, lanes *h, r*; pUC8-9, lanes *i, s*; pUC8-5, lanes *j, t*. The number above each lane refers to the position of the base substitution in the *ctc* promoter region of the template. The asterisks denote the position of the 95-base run-off transcript generated by $E\sigma^{37}$ or $E\sigma^{29}$ as indicated. The transcripts were visualized by autoradiography after electrophoresis in a 7 M urea/9% (w/v) polyacryalamide gel.

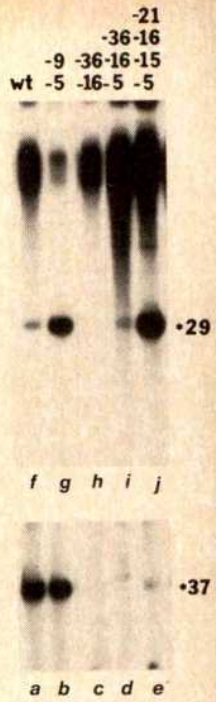


Fig. 3 Run-off transcripts from mutant promoters. RNA was transcribed with $E\sigma^{37}$ (lanes *a–e*) or $E\sigma^{29}$ (lanes *f–j*) from the following plasmid templates that had been cleaved at the *Hin*dIII site 95 bp downstream from the *ctc* promoter: pUC8-31, lanes *a, f*; pUC8-313, lanes *b, g*; pUC8-3111, lanes *c, h*; pUC8-3118, lanes *d, i*; pUC8-3146, lanes *e, j*. The number above each lane refers to the positions of the base substitutions in the *ctc* promoter region of the template (see Table 1). The asterisks denote the position of the 95-base run-off transcripts generated by $E\sigma^{37}$ or $E\sigma^{29}$ as indicated. The transcripts were visualized by autoradiography after electrophoresis in a 7 M urea/9% (w/v) polyacrylamide gel.

1, Fig. 3). Promoter 3146, containing base substitutions at positions −21, −16, −15 and −5, directed little or no transcription by $E\sigma^{37}$ (Fig. 3, lane *e*), but directed transcription by $E\sigma^{29}$ more efficiently than did the wild-type *ctc* promoter (Fig. 3, lane *j*). Promoter 313, with transitions at positions −9 and −5, was also used more efficiently by $E\sigma^{29}$ than the wild-type *ctc* promoter (Fig. 3, lane *g*). Two promoters (3111 and 3118), containing multiple base substitutions including positions −36 and −16, were used less efficiently than the wild-type *ctc* promoter by both $E\sigma^{37}$ and $E\sigma^{29}$ (Fig. 3, lanes *c, d, h, i*).

Although $E\sigma^{37}$ and $E\sigma^{29}$ use the *ctc* promoter to initiate transcription at or near the same position[5,8], the present results support a model in which these two forms of RNA polymerase utilize the *ctc* promoter by interacting with different nucleotides within the *ctc* promoter region. The most dramatic demonstration that a single base pair within the promoter is critical for utilization by one form of RNA polymerase, but not the other, is the base substitution at position −15. This base substitution severely limits utilization of the promoter by $E\sigma^{37}$, but not $E\sigma^{29}$. Promoter 3146, which has multiple base substitutions, also demonstrates the differential effects of base substitutions on promoter utilization by $E\sigma^{37}$ and $E\sigma^{29}$.

Can we identify those sequences that signal recognition by each type of RNA polymerase? We have proposed that $E\sigma^{37}$

Table 1 Activity of mutant *ctc* promoters

Plasmid	Positions of base substitutions (transitions)	Transcription relative to wild-type *ctc* promoter (%)				
		Expt 1 ($E\sigma^{29}$)	Expt 2 ($E\sigma^{29}$)	Expt 3 ($E\sigma^{29}$)	Average ($E\sigma^{29}$)	Average of 3 expts ($E\sigma^{37}$)
pUC8-31	Wild type	100	100	100	100	100
pUC8-39	−39, +61	76	85	73	78	110
pUC8-37	−37	67	40	31	46	68
pUC8-36	−36	57	16	21	31	18
pUC8-21	−21, +61	150	130	88	120	95
pUC8-16	−16, +65	30	18	57	35	31
pUC8-15	−15	270	76	140	160	16
pUC8-14	−14, +38	160	68	130	120	60
pUC8-9	−9, +51, +65	910	180	330	470	80
pUC8-5	−5	100	61	140	100	120
pUC8-313	−9, −5, +27, +39, +50, +57, +66	900	1,500	690	1,000	170*
pUC8-3146	−21, −16, −15, −5, +20, +23, +65	1,800	690	500	1,300	22*
pUC8-3111	−36, −16, +30, +66	ND	ND	ND	ND	ND
pUC8-3118	−36, −16, −5, +32	19	19	51	30	ND

Activity was determined by densitometric tracing of autoradiographs. ND, not detected.
*Average of two experiments.

recognizes its cognate promoters by interaction with the nucleotides near the −10 region (10 bp upstream from the startpoint of transcription) and the −35 region (35 bp upstream from the startpoint of transcription) of these promoters[6]. The nucleotide sequence GG-ATTG-T and AGG-TT (non-transcribed strand shown) at the −10 region and −35 region, respectively, are conserved among promoters recognized by $E\sigma^{37}$ (see Fig. 1), and methylation protection experiments reveal that these sequences are in close contact with bound $E\sigma^{37}$ (ref. 9). Finally, the base substitutions that severely affect utilization of the *ctc* promoter by $E\sigma^{37}$ alter bases at positions −36, −16, −15 and −14 (ref. 6).

The sequences CATATT-T and A-TT-AAAA (non-transcribed strand shown) at the −10 and −35 region, respectively, are highly conserved among the four promoters known to be used by $E\sigma^{29}$ (Fig. 1)[8]. The base substitutions affecting the utilization of the *ctc* promoter by $E\sigma^{29}$ (positions −36, −16 and −9) do not replace conserved nucleotides, but may act by affecting $E\sigma^{29}$ contacts with neighbouring conserved nucleotides.

A base substitution at position −36 or −16 decreases utilization of the *ctc* promoter by both $E\sigma^{29}$ and $E\sigma^{37}$, whereas other mutations differentially affect utilization of the promoter. We therefore conclude that these two RNA polymerases utilize the *ctc* promoter by interacting with two different but overlapping sets of nucleotides. According to this model, the *ctc* promoter region is a composite of two overlapping promoters, one set of nucleotides signalling recognition by $E\sigma^{37}$ and an intercalating set signalling recognition by $E\sigma^{29}$.

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1. Losick, R. & Pero, J. *Cell* **25**, 582–584 (1981).
2. Johnson, C. W., Moran, C.P. Jr & Losick, R. *Nature* **302**, 800–804 (1983).
3. Losick, R. in *Sporulation and Germination* (eds. Levinson, H. S., Sonenshein, A. L. & Tipper, D. J.) 48–56 (American Society for Microbiology, Washington DC, 1981).
4. Wong, H. C., Schnepf, H. E. & Whiteley, H. R. *J. biol. Chem.* **158**, 1960–1967 (1983).
5. Haldenwang, W. G., Lang, N. & Losick, R. *Cell* **23**, 615–624 (1981).
6. Tatti, K. M. & Moran, C. P. Jr *J. molec. Biol.* **175**, 285–297 (1984).
7. Vieira, J. & Messing, J. *Gene* **19**, 259–268 (1982).
8. Lang, N. thesis, Harvard Univ. (1982).
9. Moran, C. P. Jr, Johnson, W. C. & Losick, R. *J. molec. Biol.* **162**, 709–713 (1982).

Control of germ cell nuclear behaviour at fertilization by *Tetrahymena* intermediate filament protein

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Intermediate filament protein [relative molecular mass (M_r) 49,000 (49K)] from the ciliated protozoan *Tetrahymena* has been shown to resemble intermediate filament proteins from mammalian cells in several respects[1], and to have a possible role in the oral morphogenesis preceding binary fission in *Tetrahymena*[2]. Here, based on immunofluorescence localization of the 49K protein in *Tetrahymena* during the early stages of conjugation, we suggest that the protein is involved in some nuclear events[3–5], such as the production of four haploid nuclei by prezygotic divisions (meiosis), selection of one of the four meiotic products, formation of the gametic pronucleus by the mitotic division of the selected meiotic product, transfer of the gametic pronucleus across a cell–cell junction, and zygote formation by pronuclear fusion.

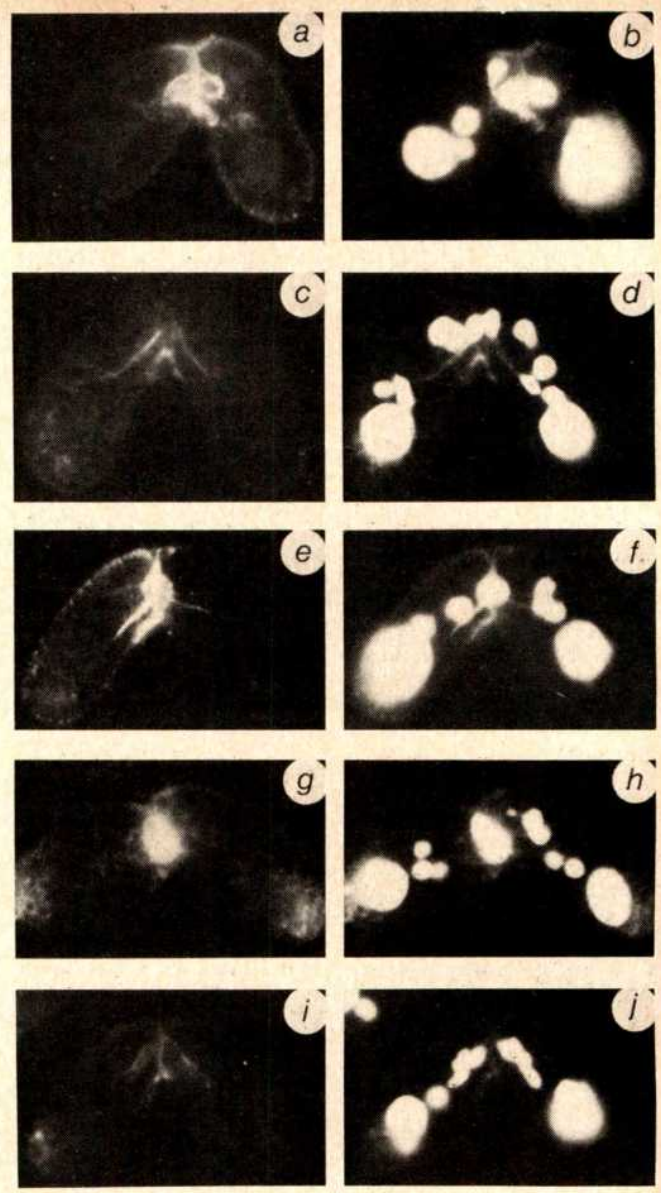


Fig. 1 Immunofluorescence localization of 49K protein in relation to the nuclear events of fertilization in *Tetrahymena*. The conjugation of *Tetrahymena thermophila* (BII and BIII) was synchronized by using a modification of the method of Sugai and Hiwatashi[14]. Detergent-extracted cells with conjugating pairs were prepared as described by Goodenough[6] and air-dried on slides. These unfixed cells were subjected to indirect immunofluorescence staining with anti-49K protein antiserum (diluted 1:160 in phosphate-buffered saline, PBS) and rhodamine-conjugated goat anti-rabbit IgG (diluted 1:200 in PBS; Miles). The macro- and micronuclei were stained with 0.5 μg ml^{-1} DAPI. *a, c, e, g, i*, Fluorescence photomicrographs of extracted cells stained with anti-49K protein antiserum. *b, d, f, h, j*, Extracted cells stained with anti-49K protein antiserum and DAPI (double exposure). *a* and *b* were taken at 5.75 h, *c* and *d* at 6.5 h, *e* and *f* at 7.0 h, *g* and *h* at 7.25 h, and *i* and *j* at 7.5 h after mixing of complementary mating-type cells. For an explanation of the dotted fluorescence in the cortical regions, see ref. 2.

By using immunofluorescence, we localized the 49K protein in *Tetrahymena* cells at various stages of synchronous conjugation, using an antiserum against 49K protein. Immune blotting experiments have shown that the antiserum is highly specific for 49K protein[2]. Using the monospecific antiserum, we observed that after pair formation in *Tetrahymena*, immunofluorescence appeared in some filamentous structures and/or in an eyeglass-like structure around the cell–cell junction. We were interested

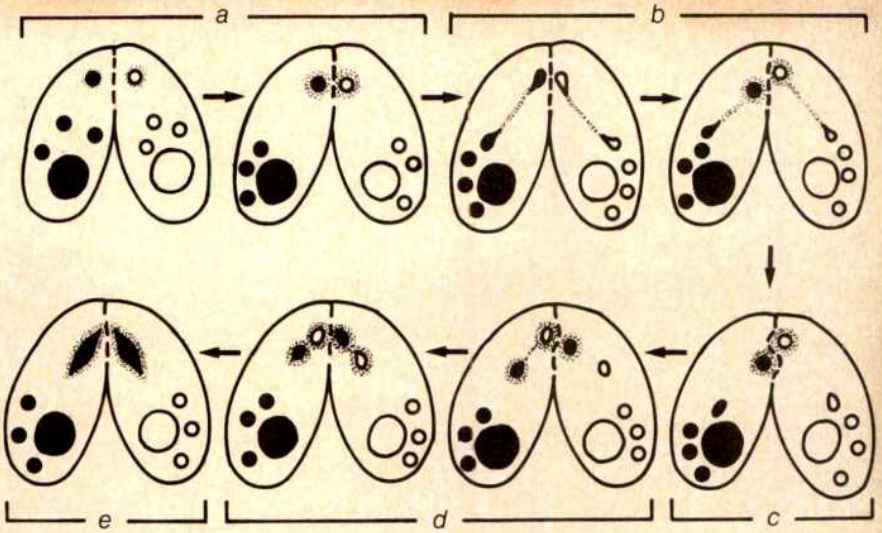


Fig. 2 Schematic diagram of the change in the distribution of 49K protein at conjugation in *Tetrahymena*: *a*–*e* represent the stages of conjugation. *a*, Only one of four meiotic products approaches the junction of the two cells (see Fig. 1*a*, *b*). *b*, This product divides mitotically to yield the migratory and stationary pronuclei (see Fig. 1*c*–*f*). *c*, The migratory pronucleus moves across the cell junction (refer to Fig. 1*g*, *h*). *d*, The stationary pronucleus moves towards the incoming migratory pronucleus (Fig. 1*i*, *j*). *e*, Two gametic pronuclei fuse to generate a fertilization nucleus. The fine black dots show the localization of the 49K protein.

in these fluorescence patterns because they seemed to reflect the behaviour of germ cell nuclei at conjugation. Because the behaviour of the nucleus can be considered as a form of cell motility and since the 49K protein is thought to constitute the cytoskeleton responsible for cell motility, immunofluorescence staining was performed on detergent-extracted cells prepared by the method of Goodenough[6]. The fluorescence patterns of these extracted cells, taken from various stages of conjugation, were essentially the same as those of whole cells, a finding that suggests strongly the importance of cytoskeletal 49K protein. Furthermore, cytoskeletal 49K protein and germ cell nuclei are located close to each other, as revealed by extracted cells double-stained with rhodamine-conjugated antibody and DAPI(4′,6-diamidino-2-phenylindole dihydrochloride) (see Fig. 1).

Next, we determined the sequence of the stages showing the various immunofluorescence patterns by measuring the frequency of occurrence of each stage (data not shown); fluorescence in the functional oral apparatus disappeared 2 h after mixing of the complementary mating-type cells. The first and second prezygotic divisions (meiosis) occurred ~4 h and 4.5 h, respectively, after mixing, and resulted in the production of four haploid nuclei. At a later stage of the second prezygotic division, filamentous structures linking the separating nuclei showed transitory fluorescence (results to be published elsewhere). At 5.75 h after mixing, one of the four meiotic products had approached the junction of the two cells; the periphery of this product fluoresced strongly, whereas the peripheries of the other three products showed no fluorescence (Fig. 1*a*, *b*). The former is known to become the functional germ nucleus and the latter three the relic nuclei. At 6.5 h, only the functionally active nucleus divided mitotically to give rise to the migratory and stationary gametic pronuclei. At the separation stage of mitosis, a filamentous structure linking the two pronuclei fluoresced conspicuously (Fig. 1*c*, *d*); immediately after this, the periphery of the presumptive migratory pronucleus fluoresced again and the filament between the pronuclei began to show diminished fluorescence (Fig. 1*e*, *f*). At 7.0 h after mixing, the migratory pronucleus was about to move across the cell junction; intense fluorescence was observed around the migratory pronucleus, whereas fluorescence from the filamentous structure disappeared (Fig. 1*g*, *h*). At 7.25 h, corresponding to the stage just after pronuclear exchange but before pronuclear fusion, a filamentous structure linking the incoming migratory nucleus and the stationary nucleus fluoresced again (Fig. 1*i*, *j*) and the peripheries of the gametic pronuclei also fluoresced. At this stage, we found a remarkable difference between the stationary pronucleus and the relic nuclei in terms of immunofluorescence. The filamentous structure shortened as the pre-fusion process progressed. At 7.5 h after mixing, the gametic pronuclei fused to form the fertilization nucleus.

It has long been thought that in *Tetrahymena* and *Paramecium*, the determination, differentiation and behaviour of germ nuclei during conjugation result from localized differences in the cytoplasm surrounding them[3,7–10], but there is no evidence of such cytoplasmic controlling factors. The present study provides some important clues for solving this problem: *Tetrahymena* intermediate filament protein (49K protein) is presumably involved in the germ nuclear events (see Fig. 2, which summarizes the results of the present experiments schematically). From the immunofluorescence localization of 49K protein, we believe that the protein is involved to some extent in: (1) the selection of one functional nucleus from four haploid nuclei generated by meiosis (Fig. 2*a*); (2) the division of the functional nucleus to generate migratory and stationary pronuclei (Fig. 2*b*); (3) the determination of the migratory pronucleus (Fig. 2*b*); (4) the migratory pronuclear exchange (Fig. 2*c*); (5) the selection of two gametic pronuclei from five nuclei (including three relic nuclei) to form the fertilization nucleus (Fig. 2*d*); and (6) the fusion of gametic pronuclei (Fig. 2*d*, *e*).

It is known that gametic pronuclear exchange is blocked by inhibitors of microtubule assembly[11]. Recently, Orias *et al.*[12] showed that each migratory pronucleus is surrounded by a microtubular meshwork. We speculate that, as well as the microtubular meshwork, the cytoskeletal 49K protein has a crucial role in the transfer of gametic pronuclei across the cell junction, and that 49K protein interacts with the microtubules. As the microtubules are observed at the time of separation of the germ pronuclei (T. Yasuda, personal communication), the 49K protein may also be present owing to its association with the microtubules. Such an association does not, however, hold true for all the cases in which microtubules are present in large numbers: ciliary axonemes, most parts of the oral apparatus, cortical microtubular bands, and the micronucleus at the crescent stage of meiotic prophase[13] showed no anti-49K protein immunofluorescence. Thus, we conclude that *Tetrahymena* intermediate filament protein may have crucial roles in fertilization either alone or in association with some microtubules having specialized functions.

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1. Numata, O. & Watanabe, Y. *J. Biochem., Tokyo* **91,** 1563–1573 (1982).
2. Numata, O., Hirono, M. & Watanabe, Y. *Expl Cell Res.* **148,** 207–220 (1983).
3. Nanney, D. L. *Biol. Bull.* **105,** 133–148 (1953).
4. Ray, C. Jr *J. Protozool.* **3,** 88–96 (1956).
5. Elliott, A. M. in *Biology of* Tetrahymena (ed. Elliott, A. M.) 259–286 (Dowden, Hutchinson & Ross, Stroudsburg, Pennsylvania, 1973).
6. Goodenough, U. W. *J. Cell Biol.* **96,** 1610–1621 (1983).
7. Sonneborn, T. M. *Caryologia* **1,** 307–325 (1954).

8. Mikami, K. *Devl Biol.* **80**, 46–55 (1980).
9. Mikami, K. *J. Cell Sci.* **56**, 453–460 (1982).
10. Mikami, K. *Expl Cell Res.* **144**, 25–30 (1983).
11. Hamilton, E. P. & Suhr-Jessen, P. B. *Expl Cell Res.* **126**, 391–396 (1980).
12. Orias, J. D., Hamilton, E. P. & Orias, E. *Science* **222**, 181–184 (1983).
13. Wolfe, J., Hunter, B. & Adair, W. S. *Chromosoma* **55**, 289–308 (1976).
14. Sugai, T. & Hiwatashi, K. *J. Protozool.* **21**, 542–548 (1974).

Reversible cyclic AMP-dependent change in distribution of myosin thick filaments in *Dictyostelium*

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Myosin is thought to act as a major mechanochemical transducer in non-muscle cell motility[1], but the *in situ* organization of the molecules has not yet been determined. Here we report the localization of myosin 'rods', analogous to the thick filaments of muscle, by ameliorated immunofluorescence and demonstrate the dynamic translocation of these rods in response to exogenously added cyclic AMP, which is a chemoattractant for *Dictyostelium* amoebae. On addition of cyclic AMP, we observed instantaneous shedding of the endoplasmic myosin followed by an increase in cortical rods, the original distribution being recovered in a few minutes. We conclude that myosin filaments mediate *Dictyostelium* cell movement, probably by an assembly/disassembly cycle of the molecules in response to a chemotactic stimulus.

We established a hybridoma that produces a monoclonal antibody (IgG) against *Dictyostelium* myosin. This antibody recognizes myosin heavy chain, using immunoblotting techniques[2]. Immunogold staining of purified *Dictyostelium* myosin shows that the antibody is reactive to reconstituted myosin thick filaments (Fig. 1*a*, *b*). We have modified our 'agar-overlay' immunofluorescence technique, which enables us to localize *Dictyostelium* actin, myosin and tubulin[2,3]. Our improved fixation protocol allows the specific visualization of fluorescent rod-like shapes (Fig. 1*c*). The rods are uniform in size (0.1–0.2 μm wide, 0.7 μm long) and identical to those found with immunofluorescent staining of reconstituted thick filaments of *Dictyostelium* myosin (Fig. 1*d*). The presumptive myosin rods are localized at the cortical region of vegetative cells, shown by fluorescence micrographs taken at several focal planes (Fig. 2*a*–*d*).

We believe that our fixation procedure reflects the *in situ* form of the molecules as: (1) the samples were fixed instantaneously in cold methanol-containing formalin and (2) once fixed, the rods did not dissolve even when the phosphate-buffered saline (PBS) was replaced with myosin solubilizing solution (containing 0.6 M KCl). We found no thick filaments in transmission electron micrographs of thin sections of cells prepared for the immunofluorescence experiments. We next investigated the possible disruption of the thick filaments by our fixation method (Table 1). Fixation with 0.1% OsO_4 for 10 min at 4 °C destroyed the rod-like structures, leaving only weak dot-like fluorescence. Thus, non-muscle myosin thick filaments are susceptible to chemical fixation, which may be the reason for the controversy about their existence in non-muscle cells[4,5].

A vegetative cell undergoes cytokinesis every 3 h. At this stage, the cortical fluorescence mostly disappears except in part of the cleavage furrow. The myosin rods are aligned in parallel with each other, forming a circular and peripheral ring-shaped fluorescence (Fig. 2*e*–*h*). This suggests the presence of a contractile ring[6] of microfilaments at the constricting portion of the dividing cells.

When the amoebae start development, their motile activity increases. During the initial 2 h of development, myosin rods increase in number and appear in the endoplasm side by side in the cortex (Figs 2*k*; 3*a*, *c*). In polarized cells undergoing

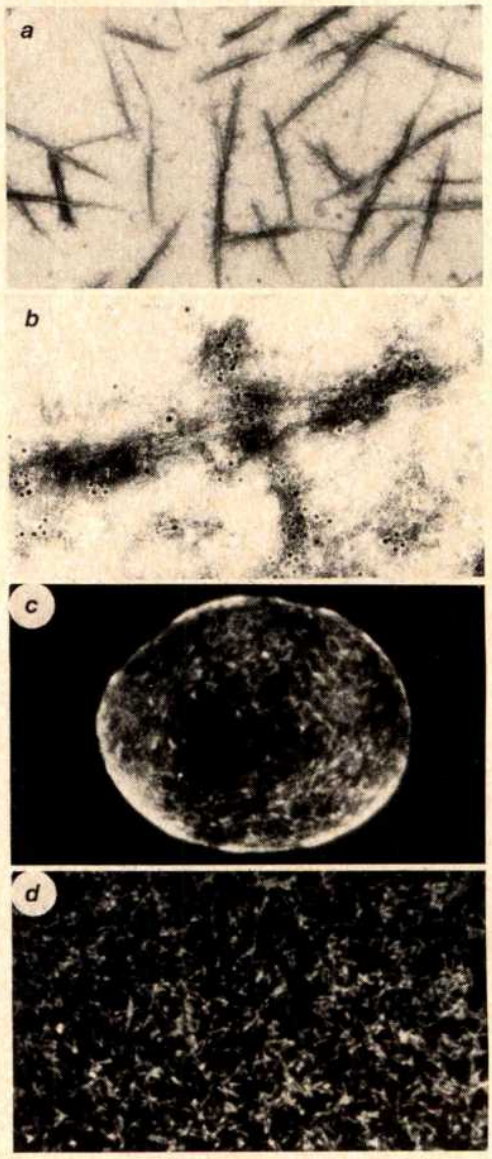


Fig. 1 Electron and immunofluorescence micrographs of reconstituted thick filaments of *Dictyostelium* myosin and *in situ* localization of presumptive thick filaments by agar-overlay immunofluorescence[2]. *a*, Negative staining of reconstituted thick filaments, ×29,600. Assembled myosin molecules form spindle-shaped bipolar filaments. *b*, Immunogold staining of purified myosin filaments, ×118,400. *c*, Immunofluorescence micrograph of a *Dictyostelium* cell prepared by agar-overlay immunofluorescence, ×1,739. *d*, Immunofluorescent staining of purified myosin thick filaments, ×1,739.

Methods. *Dictyostelium* myosin was purified by the method of Mockrin and Spudich[15] and the thick filaments formed by dialysis against a low salt buffer (50 mM KCl, 1 mM EDTA, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 10 mM imidazole, *p*H 6.5). *b*, Reconstituted thick filaments were placed on a poly-L-lysine-coated grid, incubated with our myosin antibody and stained with colloidal gold-conjugated second antibody (GAM-G5, Janssen). The monoclonal anti-*Dictyostelium* myosin binds to the thick filaments. *c*, Cells were placed on a coverslip, overlaid with a thin agarose sheet and immediately immersed into methanol containing 1% formalin at −15 °C, then fixed for 5 min. After washing with PBS, the cells were incubated with the anti-*Dictyostelium* myosin for 30 min and then fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG (Miles-Yeda) for 30 min at 37 °C. *d*, Reconstituted thick filaments were placed on a poly-L-lysine-coated coverslip, incubated with our antibody and stained with FITC-labelled second antibody.

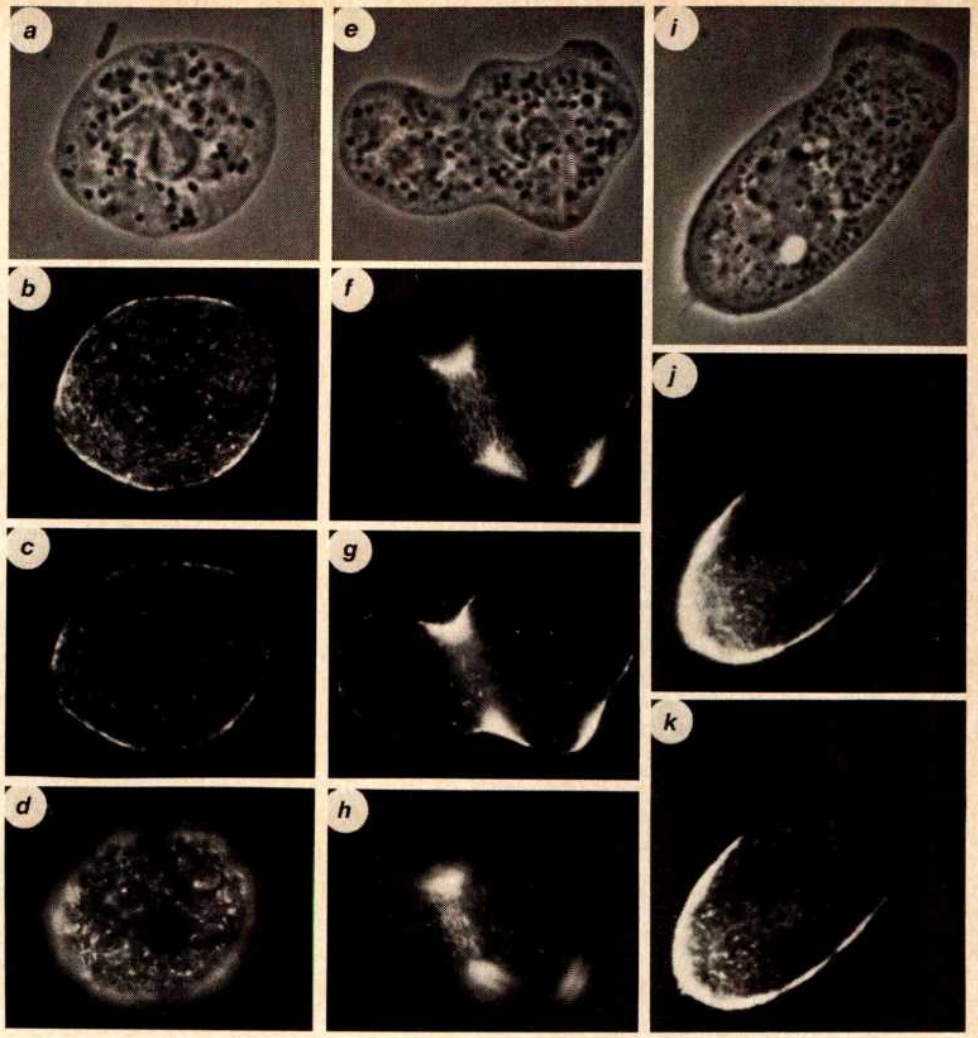


Fig. 2 Specific distribution of myosin rods in *Dictyostelium discoideum* amoebae shown by agar-overlay immunofluorescence[2]. *a–d*, Phase-contrast (*a*) and fluorescence (*b–d*) micrographs showing specific localization of myosin rods at the cortical region of a vegetative cell. ×1,572. Immunofluorescence micrographs taken at three different focal planes of the cell verified that the rods were localized only at the upper (*b*) and lower (*d*) cortices. Note that the rods are aligned in parallel with the cortex. *e–h*, Phase-contrast (*e*) and fluorescence (*f–h*) micrographs showing the distribution of myosin rods aligned in parallel forming a contractile-ring-like array. ×1,443. *f–h*, Immunofluorescence micrographs taken at three different focal planes of the cell performing cytokinesis. The micrographs taken at the upper (*f*), middle (*g*), or lower (*h*) plane confirmed that the rods were arranged in a peripheral circular ring at the cleavage furrow. The number of rods at the cell cortex other than the constricting portion have decreased. *i–k*, Phase-contrast (*i*) and immunofluorescence (*j*, *k*) micrographs showing that the myosin rods are localized specifically in the posterior cortex of a polarized cell and form linear alignments. *j*, *k*, Immunofluorescence micrographs taken at the upper (*j*) or middle (*k*) plane demonstrate that the rods are localized in the endoplasm (*k*) as well as in the cortex (*j*) at this stage. ×1,572.

Table 1 Effects of chemical fixation on the disruption of *in situ* myosin rods of *Dictyostelium*

Glutaraldehyde %	OsO_4 %	Myosin rods
0	0	+
1	0	?
2	0	−
0	0.01	+
0	0.1	??
1	0.01	−
2	0.1	−

A stock solution of glutaraldehyde (25%; Electron Microscopy Science) was diluted with PBS (138 mM NaCl, 2.7 mM KCl, 8 mM phosphate buffer, *p*H 7.2). The sample was fixed for 30 min at 25 °C. Stock solution of OsO_4 (8%; Merck) was diluted with PBS and the sample fixed for 10 min at 4 °C. The degree of fluorescent staining of myosin rods was judged as follows: −, no specific rod-like fluorescence detectable, partially because of autofluorescence from glutaraldehyde; +, normal rod-like fluorescence; ?, faint rod-like fluorescence identified against the bright autofluorescence; ??, fluorescent dots were seen instead of rod-like shapes.

directed locomotion with the anterior pseudopod, we find that the rods accumulate in the posterior cortex. Occasionally, some near alignments of the rods occur just beneath the cortex (Fig. *i–k*). This evidence supports the suggestion that the force for moeboid movement is generated by contraction of the posterior cortex[2,7].

The assembly of *Dictyostelium* myosin is thought to be regulated by heavy-chain phosphorylation; dephosphorylation would cause the monomeric myosin to form thick filaments with much actin-activated ATPase[8], in contrast to the situation in mammalian myosins[9,10]. Malchow *et al.*[11] found that the addition of the chemoattractant cyclic AMP to the aggregation-competent cells induced the rapid and transient phosphorylation/dephosphorylation cycle of myosin heavy chains. Do thick filaments perform cyclic changes of disassembly/assembly in response to the cyclic AMP signal? Here, we find that the myosin rods disappear from the endoplasm and accumulate in the ectoplasm within 2 min of treatment with 10^{-6} M cyclic AMP at 5 °C. After 1 min, the rods have redistributed throughout the endoplasm (Fig. 3*a–c*). This change in distribution is dramatic and occurs in almost all of the observed cells within 1 min after the addition of cyclic AMP at 11 °C; it is too fast to follow at 23 °C.

If cyclic AMP is applied locally, the cell extends a pseudopod in the direction of the signal within several seconds[12]. Our results described here suggest that translocation of the endoplasmic myosin rods is coincident with this response to a chemotactic stimulus. As the accumulation of rods in the cell cortex is transient, we asked whether a cell without these endoplasmic rods could contract. We prepared Triton-insoluble ghosts of cells with endoplasmic rods depleted by treatment with cyclic AMP and examined their contractility in response to ATP. SDS-gel electrophoresis shows that the ghosts are composed mainly of actin and myosin, comprising up to 25% and 50% of the total cellular proteins (Fig. 3*f*). When 1 mM ATP is added to the ghosts, the cortex contracts in a few seconds (Fig. 3*d*, *e*). This suggests that the cortical myosin rods are organized in such a way to generate the force required for amoeboid movement.

The myosin rods described here are much larger than found normally in non-muscle cells[4]. We believe that the rod-shaped

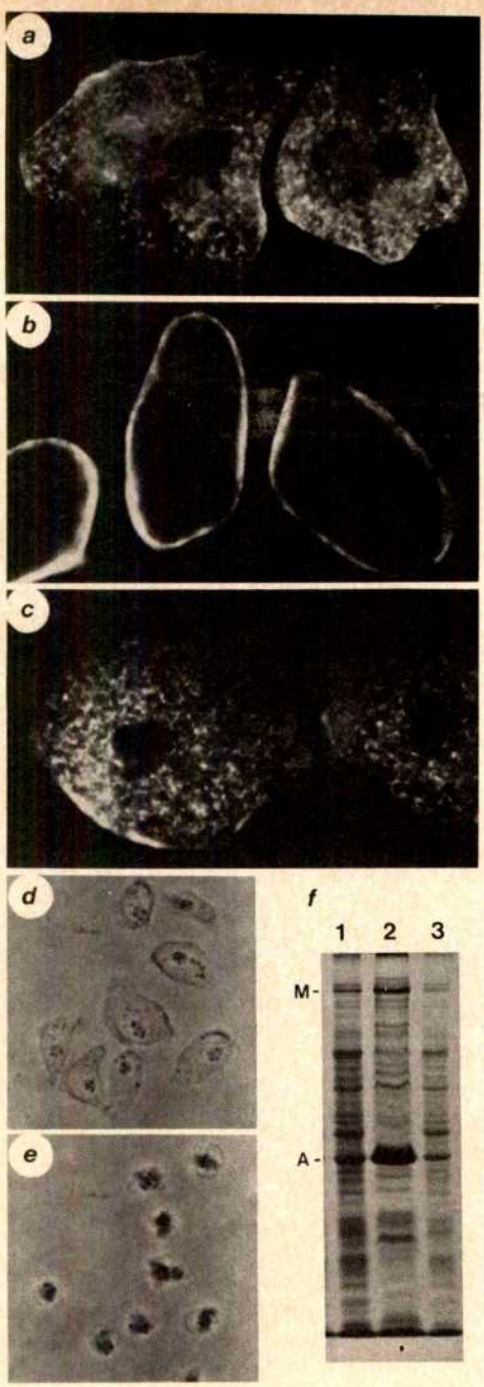


Fig. 3 Changes in the distribution of myosin rods in response to exogenously added cyclic AMP and the contractility of the cell cortex. A 10^{-6} M cyclic AMP solution in PBS buffer (15 mM, *p*H 6.5) was added to aggregation-competent cells overlaid with a thin agarose sheet at 5 °C. After the indicated time, the cells were plunged into −15 °C methanol containing 1% formalin and prepared for the agar-overlay immunofluorescence[2]. *a*, Control cell; *b*, 2 min; *c*, 3 min after cyclic AMP application ×1,350. Note the disappearance of the endoplasmic rods and their accumulation at the cortex in *b* and their reappearance in *c*. The same batch of cells was overlaid with a thin agarose sheet and treated with 10^{-6} M cyclic AMP at 5 °C. After 2 min, when the endoplasmic rods disappeared as shown in *b*, the cells were permeabilized with P buffer (10 mM PIPES, 50 mM KCl, 1 mM $MgCl_2$, 10 mM EGTA, 1 mM DTT, 0.2 mM phenylmethylsulphonyl fluoride, *p*H 7.6) containing 0.5% Triton X-100 for 10 min at 0 °C, then extracted with P buffer for 5 min. Phase-contrast micrograph (*d*) shows that the Triton-insoluble ghosts contain the cell cortex, nucleus and some cytoplasmic debris. *d*, *e*, Contracted ghosts a few seconds after application of P buffer containing 1 mM ATP. ×417. For SDS-polyacrylamide gel electrophoresis, a suspension of aggregation-competent cells was treated with 10^{-6} M cyclic AMP for 2 min at 5 °C, then permeabilized with P buffer containing 0.5% Triton X-100 for 10 min on ice. After 5 min centrifugation at 10,000 r.p.m. the pellet and supernatant were separated and boiled for a few minutes in SDS sample buffer (0.25 M Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, *p*H 6.8). The whole-cell lysate was prepared by solubilizing the cells with pre-warmed SDS sample buffer. SDS-polyacrylamide gel electrophoresis was performed[16] and protein quantity was estimated using a Gelman Model DCD-16 densitometer. *f*, Lane 1, whole-cell lysate (100 μg); lane 2, Triton-insoluble proteins (85 μg); lane 3, Triton-soluble proteins (50 μg); A, actin; M, myosin heavy chain.

fluorescence represents *in situ* myosin thick filaments, partly because of their immunological and morphological characteristics, and partly because of their specific localization in the cleavage furrow, their linear alignment and the dynamic cycle of the possible disassembly/assembly in response to cyclic AMP. The rods observed by immunofluorescence seem larger than those observed by negative stain (Fig. 1*a*, *d*). The size is probably amplified both by the antibody binding and by the fluorescence reflecting fuzzy, unwound projections of the molecules radiating from the rods.

An average cell should contain $\sim 3.8 \times 10^{-10}$ mg myosin, assuming that each cell has 7.5×10^{-8} mg protein and that myosin is 0.5% of the total cell protein[13], equivalent to 5×10^5 myosin molecules. Assuming that ~75% of the molecules formed thick filaments (suggested by *in vitro* phosphorylation[8]) and that each thick filament is composed of 30 molecules, one cell should contain $\sim 1.3 \times 10^4$ thick filaments. This value is in agreement with the numbers counted here (Fig. 1*c*). We speculate that these rods are transferred by changes in the molecular assembly of myosin, possibly regulated by the phosphorylation/dephosphorylation of the molecules coupled with the chemotactic response. It is reasonable to suppose that the return of cortical rods to the endoplasm results from ATP-dependent contraction of cortical actomyosin. This idea is supported by the fact that *Dictyostelium* myosin bound to the membrane preparation is released from the cell cortex on treatment with Mg-ATP[14].

We conclude that *Dictyostelium* myosin forms thick filaments *in situ* and that translocation of these filaments occurs in coordination with the assembly/disassembly of the molecules in response to physiological changes.

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1. Clarke, M. & Spudich, J. A. *A. Rev. Biochem.* **46**, 797–822 (1977).
2. Yumura, S., Mori, H. & Fukui, Y. *J. Cell Biol.* **99**, 894–899 (1984).
3. Kitanishi, T., Shibaoka, H. & Fukui, Y. *Protoplasma* **120**, 185–196 (1984).
4. Hirokawa, N., Tilney, L. G., Fujiwara, K. & Heuser, J. E. *J. Cell Biol.* **94**, 425–443 (1982).
5. Herman, I. M. & Pollard, T. D. *J. Cell Biol.* **88**, 346–351 (1981).
6. Schroeder, T. E. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1688–1692 (1973).
7. Hellewell, S. B. & Taylor, D. L. *J. Cell Biol.* **83**, 633–648 (1979).
8. Kuczmarski, E. D. & Spudich, J. A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7292–7296 (1980).
9. Suzuki, H., Onishi, H., Takahashi, K. & Watanabe, S. *J. Biochem., Tokyo* **84**, 1529–1542 (1978).
10. Sholey, J. M., Taylor, K. A. & Kendrick-Jones, J. *Nature* **287**, 233–235 (1980).
11. Malchow, D., Böhme, R. & Rahmsdorf, H. J. *Eur. J. Biochem.* **117**, 213–218 (1981).
12. Gerisch, G. *et al.* in *Developmental Biology* (eds McMahon, D. & Fox, C. F.) 76–88 (Benjamin, Menlo Park, 1975).
13. Clarke, M. & Spudich, J. A. *J. molec. Biol.* **86**, 209–222 (1974).
14. Spudich, J. A. *J. biol. Chem.* **249**, 6013–6020 (1974).
15. Mockrin, S. C. & Spudich, J. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2321–2325 (1976).
16. Laemmli, U. K. *Nature* **227**, 680–685 (1970).

Association of histone H1^0 with a gene repressed during liver development

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Histone H1^0 has a number of unusual properties that set it apart from other H1 subtypes (for review see ref. 1). For example, H1^0 synthesis is not strictly coupled to DNA synthesis[2], it is absent from the embryonic liver of mice (but present shortly after birth)[3,4] and its synthesis is hormone-dependent in some of the glands of adult rodents[3]. All the H1 subtypes differ in their DNA binding properties, and H1^0 has been shown to be preferentially associated with nuclease-resistant chromatin[5]. These features suggest that the H1^0 may have a role in developmental gene control. To investigate this further, we have fractionated the H1^0-containing nucleosomes of chromatin from adult mouse liver. We report here that the gene for α-fetoprotein, which is expressed in embryonic tissue but repressed soon after birth[6–9], is preferentially associated with the H1^0-containing nucleosomes. The related gene for albumin, which is expressed in both embryonic and adult tissues, is absent from the H1^0-containing nucleosome fraction. These results support a role for histone H1^0 in the control of gene expression.

Varshavsky[10] and Smith and Johns[11] have demonstrated that nucleosomes can be separated as a function of their H1 content by a non-denaturing polyacrylamide gel electrophoresis system[12]. We have adapted this technique for the preparation of H1^0-containing nucleosomes using a digest of adult mouse liver nuclei in which the amount of H1^0 relative to total H1 is 35%. As shown in Fig. 1, monomers representing 50% of the total DNA in the conditions of digestion can be resolved into four bands. To characterize the monomers further, the bands were excised from the gel and their protein content and DNA length analysed. The results are shown in Fig. 1 and summarized in Table 1.

Class 1 and 2 monomers contain no histone H1; class 3 monomers contain histone H1^0 only, with a slight contamination by H1-2 (<1%), while class 4 monomers contain the full complement of histone H1 (H1-1, H1-2, H1^0). The amount of H1^0 relative to total H1 is 20%, indicating that class 4 monomers contain ~20% of nucleosomes bearing H1^0 if one assumes one molecule of H1 per nucleosome.

The DNA length measurements show that class 1 monomers are actually core particles, while class 2, 3 and 4 monomers have the DNA length expected for nucleosomes, 166–204 base pairs. DNA from the various monomer fractions was purified as described in Fig. 1 legend and spotted in equal amounts on nitrocellulose filters. The filters were hybridized either with a probe for albumin gene (ALB) or with a probe for α-fetoprotein gene (AFP), that is, genes that are, respectively, expressed and not expressed in adult mouse liver.

The DNA of the nucleosome fraction containing only H1^0 (class 3 monomers) hybridizes strongly with the AFP probe but very weakly with the ALB probe (Fig. 2). In contrast, the DNA of class 1 and 4 monomers hybridizes strongly with the ALB probe but very weakly with the AFP probe (Fig. 2). The DNA purified from unfractionated nucleosomes hybridizes roughly equally with both probes. The fractionation procedure used in our study produces two populations of H1^0-containing nucleosomes that may reflect heterogeneity in H1^0 (ref. 13). The 20% of H1^0-containing nucleosomes in class 4 monomers do not hybridize to the AFP probe, which suggests that their functional properties differ from those of the H1^0-containing nucleosomes of class 3 monomers.

The present study shows that: (1) A coding sequence expressed in adult liver (ALB) is found in the fraction of class 4 monomers which contains the full complement of H1 histones. The results concerning the presence of H1 in active chromatin are still unclear, but our observation agrees with Berent and Sevall[14] and with Weintraub[15], who have shown that H1 is present in active chromatin. (2) Most of a coding sequence not expressed in the adult liver (AFP) is found in the fraction of monomers containing only H1^0, while an expressed coding sequence (ALB) is absent from this fraction but found in the

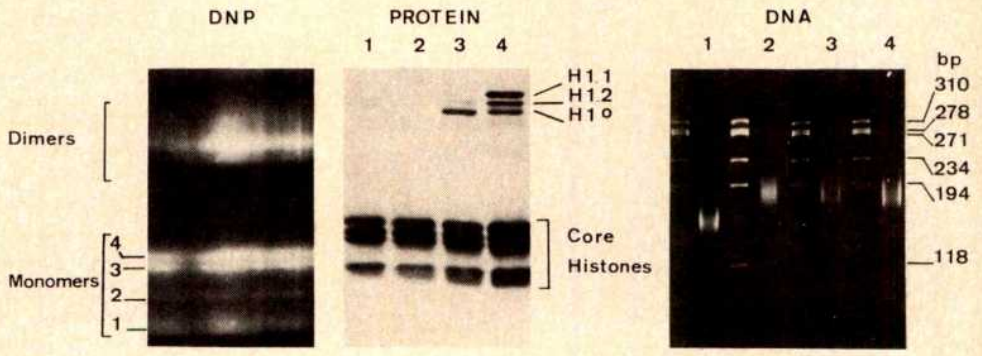


Fig. 1 DNP particle (deoxyribonucleoprotein particle) gel electrophoresis. Adult mouse liver nuclei, prepared as described elsewhere[16], were digested with micrococcal nuclease (200 U nuclease per 10^8 nuclei, 10 min at 37 °C). The digested nuclei were lysed in 0.2 mM EDTA *p*H 7, containing 1 mM phenylmethylsulphonyl fluoride. The lysate (10 A_{260} units) was fractionated by electrophoresis on a preparative 5% polyacrylamide slab gel (15×30×0.3 cm) as described by Varshavsky *et al.*[12]. Electrophoresis was performed at 200 V for 16 h at 4 °C with a continuous circulation of the buffer between the two reservoirs. The bands were visualized after ethidium bromide staining (see text for the description of the bands). Protein content of the various nucleosomes: The protein content of the DNP gel slice was obtained after electroelution in 0.025 M Tris, 0.192 M glycine, 0.1% SDS *p*H 8.3 followed by dialysis against water and lyophilization. They were then analysed on an SDS–15% polyacrylamide gel[17] and stained with Coomassie blue. DNA length of the various nucleosomes: The gel slices from the DNP gel were cut into fine particles and incubated overnight at 20 °C in 5 vol. 0.5% SDS, 3 mM EDTA *p*H 7.6 (ref. 12). Pieces of the gel were then removed by centrifugation at 10,000*g* for 10 min followed by filtration on glass wool. The supernatant was treated first with 50 g ml^{-1} RNase T1, 1 h at 37 °C, then with 100 g ml^{-1} proteinase K, 48 h at 37 °C. The extract was made 1.5 M in NaCl, cooled to 4 °C and SDS was removed by centrifugation at 10,000*g* for 10 min. The supernatant was extracted with phenol, then with chloroform[18]. Chloroform was removed by ether extraction. The DNA was precipitated with 2 vol. ethanol for 24 h at −20 °C in the presence of 10 mM $MgCl_2$ and recovered after centrifugation at 110,000*g* for 3 h in a SW 28 rotor. The pellet was then washed with 95% ethanol and DNA reprecipitated as above. Analysis of the DNA length was performed by electrophoresis on a 6% polyacrylamide gel[19]. A *Hae*III digest of RFΦX174 was used as size marker. DNA lengths are expressed in base pairs (bp).

Table 1 H1 content and DNA length of the various monomers

Class of monomers	% Monomer (relative proportion)	H1 content	% $H1^0$/total H1	DNA length in base pairs at Mean	Halfwidth
1	35	No	—	148 ± 3	140–166
2	10	No	—	183 ± 3	169–202
3	15	$H1^0$	99	177 ± 3	166–200
4	40	H1-1, H1-2, $H1^0$	20	180 ± 3	167–204
Native chromatin		H1-1, H1-2, $H1^0$	35	—	—

$H1^0$/total H1 was determined by densitometry after scanning the protein electrophoresis gel (Fig. 1). DNA length, obtained by densitometry scanning of the DNA electrophoresis gel (Fig. 1), is expressed in base pairs at the maximum absorbance and at the halfwidth of the peak.

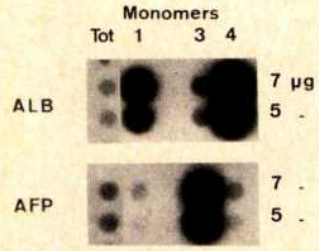


Fig. 2 Albumin and α-fetoprotein sequences among the various monomers. Purified DNAs isolated from the different monomers were hybridized to AFP1 and pmalb2 probes.

Methods. Plasmids were amplified in *Escherichia coli* (HB101) in rich medium containing 25 g ml^{-1} ampicillin and recovered by lysis of the cells with alkali[18]. They were purified by centrifugation to equilibrium in a caesium chloride/ethidium bromide density gradient and purified as described elsewhere[18]. The probes were excised by *Hin*dIII digestion[18]. They were further purified by electroelution in water after electrophoresis in 1% agarose gel in 89 mM Tris buffer *p*H 8.0, 89 mM boric acid, 2 mM EDTA and 0.025 g ml^{-1} ethidium bromide. They were then purified by chromatography on ELUTIP (Cera Labo) and precipitated with 2 vol. ethanol. The purity and the amount of DNA were estimated from the ultraviolet absorption spectra. Probes were labelled by nick-translation[18] with ^{32}P-dCTP and ^{32}P-dTTP to a specific activity of $2.2–2.5 \times 10^8$ c.p.m. per g DNA. The DNA from the various monomer fractions was dissolved in 10 mM Tris-HCl *p*H 8.0, 1 mM EDTA and denatured by heating to 100°C for 10 min. After chilling the samples on ice, an equal volume of 1 M NaOH was added and the solution was incubated at room temperature for 20 min. The DNA samples were then neutralized by adding 0.5 vol. of a solution of 1 M NaCl, 0.3 M sodium citrate, 0.5 M Tris-HCl *p*H 8.0 and 1 M HCl[18]. They were spotted directly onto nitrocellulose paper (BA 83, 0.25 µm, Schleicher & Schuell) which had been treated previously with H_2O and equilibrated with 20× SSC buffer (1×SSC buffer is 0.15 M NaCl, 0.015 M trisodium citrate) using a vacuum minifold apparatus (Schleicher & Schuell). The nitrocellulose filters were dried under a lamp and baked for 2 h at 80 °C. They were then prehybridized for 24 h at 42 °C and hybridized for 65 h at 42 °C using the technique described by Thomas[20]. The blots were exposed to X-ray film for 3 days at −70 °C using a Kodak intensifying screen. Total monomers (Tot) were prepared by two successive centrifugations on a 5–30% sucrose gradient for 21 h at 110,000*g* followed by treatment with RNase and proteinase K and purification by phenol and chloroform[18]. Equal amounts of the different DNA samples on the filters were spotted (5 and 7 µg).

other fractions tested (class 1 and 4 monomers). During maturation, the repression of the AFP gene[8,9] occurs concomitantly with the increase in $H1^0$ content[3,4]. We show here that coding sequences for the AFP gene are preferentially associated with nucleosomes bearing $H1^0$. This result reinforces the hypothesis that $H1^0$ may be involved at the level of gene expression during development.

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1. Smith, B. J., Harris, M. R., Sigournay, C. M., Mayes, E. L. V. & Bustin, M. *Eur. J. Biochem.* **138**, 309–317 (1984).
2. Chabanas, A., Lawrence, J. J., Humbert, J. & Eisen, H. *EMBO J.* **2**, 833–837 (1983).
3. Gjerset, R., Gorka, C., Hasthorpe, S., Lawrence, J. J. & Eisen, H. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2333–2337 (1982).
4. Larue, H., Bissonnette, E. & Beranger, L. *Can. J. Biochem. cell. Biol.* **61**, 1197–1200 (1983).
5. Gorka, C. & Lawrence, J. J. *Nucleic Acids Res.* **7**, 347–359 (1979).
6. Gorin, M. B. & Tilghman, S. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1351–1355 (1980).
7. Kioussis, D. *et al. J. biol. Chem.* **256**, 1960–1967 (1981).
8. Tilghman, S. M. & Belayew, A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5254–5257 (1982).
9. Nahon, J. L., Gal, A., Frain, M., Sell, S. & Sala-Trepat, J. M. *Nucleic Acids Res.* **10**, 1895–1911 (1982).
10. Varshavsky, A. J., Bakayev, V. V., Nedospasov, S. A. & Georgiev, G. P. *Cold Spring Harb. Symp. quant. Biol.* **42**, 457–473 (1978).
11. Smith, B. J. & Johns, E. W. *Nucleic Acids Res.* **8**, 6069–6079 (1980).
12. Varshavsky, A. J., Bakayev, V. V. & Georgiev, G. P. *Nucleic Acids Res.* **3**, 477–492 (1976).
13. Smith, B. J. & Johns, E. W. *FEBS Lett.* **110**, 25–29 (1980).
14. Berent, S. L. & Sevall, J. S. *Biochemistry* **23**, 2977–2983 (1984).
15. Weintraub, H. *Cell* **38**, 17–27 (1984).
16. Hewish, D. R. & Burgoyne, L. A. *Biochem. biophys. Res. Commun.* **52**, 504–510 (1973).
17. Laemmli, U. K. *Nature* **277**, 680–685 (1970).
18. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning, A Laboratory Manual* 331 (Cold Spring Harbor Laboratory, New York, 1982).
19. Maniatis, T., Jeffrey, A. & Van de Sande, H. *Biochemistry* **14**, 3787–3794 (1975).
20. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).

Corrigendum

A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene

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Nature **312**, 612–615 (1984)

THE fourth paragraph in the Discussion section of this article (p. 615) contained several inaccuracies resulting from the use of upper-case Greek alpha, which looks the same as the upper-case letter *A*. The paragraph should read:

There is evidence that other yeast genes can be repressed by negative regulatory factors bound between the upstream activator sequence and the TATA region. For example, it is likely that *MATα1* transcription is repressed in diploid yeast by binding of a negative regulatory factor (which is dependent on the expression of *MATA1* and *MATα2*) to a site between the *MATα1* upstream activator sequence and its TATA region[16]. *STE6* transcription is repressed by the *MATα2* product, which binds to a specific site in the *STE6* promoter, probably located between the upstream activator sequence and the TATA region (A. Johnson, K. Wilson and I. Herskowitz, personal communication). The repression of *MATα1* and *STE6* is at least 50 times greater than the 10-fold repression we observe for *lexA* operator-containing derivatives of the *GAL1* promoter. We do not understand why these yeast promoters are repressed so efficiently relative to the *lexA* operator-containing *GAL1* promoter.

Sequence similarity between EGF receptor and α_1-acid glycoprotein

THE human epidermal growth factor (EGF) receptor precursor consists of three domains, an extracellular EGF binding domain, a single transmembrane domain and a cytoplasmic domain. Ullrich *et al.*[1] recently determined the complete amino acid sequence of the molecule and showed that the latter two domains exhibit close similarity in sequence to the entire v-*erb-B* oncogene product, a member of the *src* family. By aligning cysteine residues, they also found repeated structures of ~170 residues within the extracellular domain[1]. A similarity matrix comparison[2], however, revealed the presence of further extensive homology between regions of ~300 residues located at positions 1-274 and 294-615, which cover almost the entire extracellular domain (Fig. 1). Of all the positions compared in the aligned sequences, 28% are occupied by identical residues (gaps were considered as substitutions between different amino acids regardless of their length). Furthermore, a statistical test[2] showed that the similarity between the aligned sequences is highly significant, with a probability of occurrence by chance of $<10^{-9}$. Thus, the extracellular EGF binding domain consists of tandemly repeated domains which may have evolved by internal duplication. Alignment of their sequences also revealed that each repeat is composed of an N-terminal conserved region and a C-terminal cysteine-rich region.

We subjected the amino acid sequence of the EGF receptor to a computer-assisted search for homology with ~2,500 known sequences compiled in our database and found apparent sequence similarity between the C-terminal half of the EGF binding domain and α_1-acid glycoprotein (α_1-AGP), known to be an acute-phase protein[3] (Fig. 1). A statistical test shows the similarity to be highly significant, with $P = 3 \times 10^{-7}$.

Interestingly, the α_1-AGP molecule has been shown to exhibit weak sequence homology to immunoglobulin[3] as well as to part of the variable domain of the mouse T-cell receptor ($P < 3 \times 10^{-4}$). Thus, it seems likely that the EGF binding domain is indirectly related to the immunoglobulin superfamily. Lack of significant similarity between the N-terminal half of the EGF binding domain and α_1-AGP may be a result of rapid divergence in the N-terminal half, relative to the C-terminal half, presumably during the earlier evolutionary stage after the separation of the two domains by internal duplication. In addition, the transmembrane segment of this receptor bears a close resemblance to that of the HLA class II β-chain ($P < 5 \times 10^{-4}$).

These results, together with evidence that the cytoplasmic domain contains an *src*-related sequence[1], suggest that the extracellular and cytoplasmic domains may have been derived from evolutionarily independent origins and thus that this large receptor gene may have evolved by gene shuffling and partial duplication. We cannot exclude the possibility, however, that an ancestral α_1-AGP gene was derived from a part of the EGF binding domain.

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1. Ullrich, A. *et al. Nature* **309**, 418-425 (1984).
2. Toh, H., Hayashida, H. & Miyata, T. *Nature* **305**, 827-829 (1983).
3. Schmid, K. *et al. Biochemistry* **12**, 2711-2724 (1973).
4. Larhammar, D., Hyldig-Nielsen, J. J., Andersson, G., Rask, L. & Peterson, P. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7313-7317 (1983).

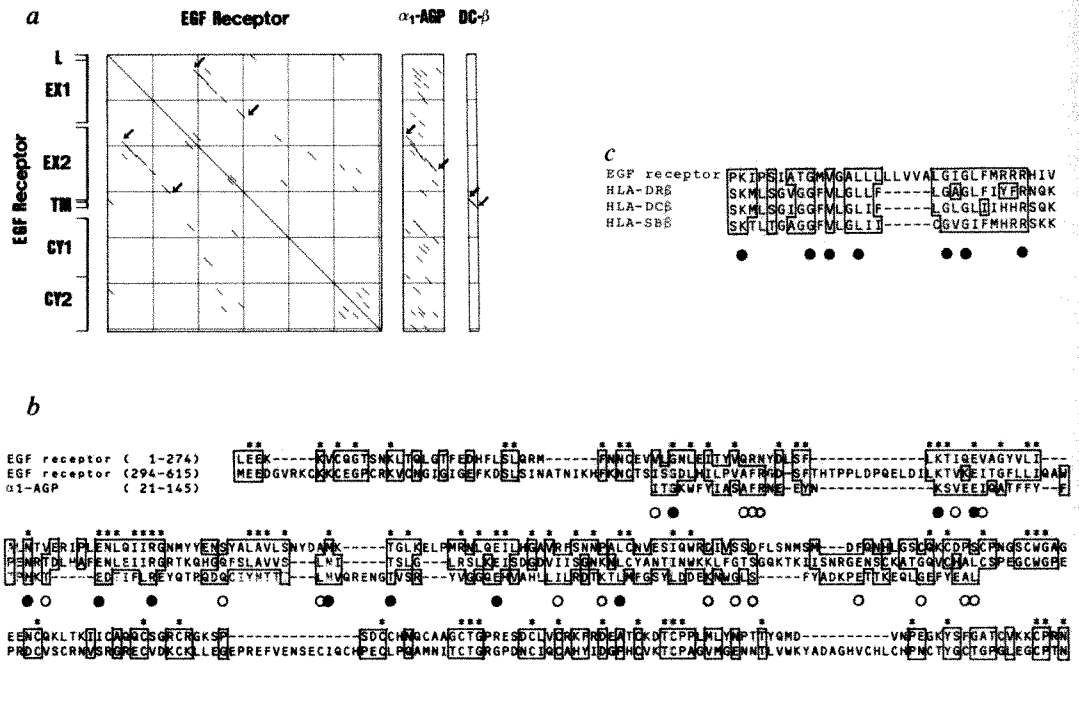


Fig. 1 *a*, Similarity matrix comparisons of amino acid sequence of EGF receptor (ordinate) with the sequences of itself, α_1-AGP and the transmembrane segment of HLA-DC β-chain (abscissa). A computer program was used to generate diagonal lines indicating segments of 25 residues which show sequence similarity (see ref. 2). L, signal peptide (positions −24 to −1); EX1, EX2, duplicated regions (1-274 and 294-615) of the extracellular EGF binding domain, respectively; TM, the transmembrane segment (622-644); CY1, CY2, *src*-homologous and -non-homologous regions (690-936 and 967-1,186) of the cytoplasmic domain, respectively. Arrows indicate regions where extensive similarities were detected. *b*, Alignment of amino-acid sequences of N-terminal (EX1) and C-terminal (EX2) halves of the EGF binding domain and α_1-AGP. Boxed residues indicate identities or favoured amino-acid substitutions (see ref. 2). Positions where residues are identical between EX1 and EX2 and between EX2 and α_1-AGP are marked by * and ○, respectively. Invariant residues among the three are indicated by ●. Gaps (—) were introduced to maximize similarity. *c*, Alignment of transmembrane segments of EGF receptor and HLA class II β-chains. Regions where sequences were aligned correspond to positions 617-650 and 197-225 of the EGF receptor and HLA-DCβ, respectively. Boxed residues indicate identities (●) or favoured amino-acid substitutions between EGF receptor and HLA chains.

Stomatal mechanism as the basis of evolution of crassulacean acid metabolism

KEELEY *et al.*[1] have demonstrated clearly that the rare pteridophyte, *Stylites andicola* (Isoetaceae), exhibits a variant of photosynthetic carbon metabolism which has obvious affinities with crassulacean acid metabolism (CAM). They also drew attention to the absence of stomata in *Stylites* and concluded that these findings taken together indicate that this unusual organism does not conform to the hypothesis that CAM may have evolved from stomatal guard cell metabolism[2].

However, as other members of the Isoetaceae possess stomata, it may be that *Stylites* evolved from ancestors that did possess stomata and although it does not itself possess these structures, it may nevertheless use pathways derived from guard cell metabolism.

Thus the discovery of a CAM-like photosynthetic mechanism in *S. andicola* is not necessarily inconsistent with the evolution of CAM and associated pathways from stomatal guard cell metabolism.

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1. Keeley, J. E., Osmond, C. B. & Raven, J. A. *Nature* **310**, 694-695 (1984).
2. Cockburn, W. *Pl. Cell Envir.* **4**, 417-418 (1981).

THE suggestion by Keeley *et al.*[1], supported by Moore[2], that *Stylites* may represent an important link between aquatic plants and primitive land plants has little foundation in fact. To go further and propose that these plants could be relics of a marginally successful compromise from which present-day terrestrial photosynthetic systems were selected runs contrary to our views on plant evolution. *Stylites*, whose separation from *Isoetes* is questionable, is regarded generally as a descendant of a long line of lycophytes and should not be thought of as primitive plants. Specifically, they most probably evolved from woody-stemmed plants with cormose bases, such as the Carboniferous swamp plant *Chaloneria*. These early lycophytes certainly possessed stomata[3]. *Isoetes*-like plants existed in the Triassic and probably spread along the coastal plains before moving inland. These are terrestrial species of *Isoetes*, shallow-water-living species (of which some have emergent sporophylls and others are totally submerged) and species living on the bottom of deep-water lakes. This migration into water resulted in the loss of stomatal function in shallow-water-living species (for example, *Isoetes malinverneana*) and the total loss of stomata in the deeper living species (for example, *Isoetes lacustris*)[4]. The most plausible argument for *Stylites* not possessing stomata is that it lost them through submergence, not that its ancestors never possessed them.

It is true that in its type locality, *Stylites* grows above the water level, but plants have been found repeatedly that grow submerged. The lack of an efficient method of dispersal suggests that the plants have been growing in the same area for a very long time. The substantial elevation of the high Andes in relatively recent geological times could explain both their geographical isolation and the loss of their aquatic habitats.

The fact that both *Stylites* undergo CAM similar to the aquatic species of *Isoetes* is therefore not necessarily surprising. *Stylites* should not be thought of as evidence that CAM did not evolve from stomatal guard cell metabolism. It is more probable that its ability to convert to CAM as it invaded an aquatic environment saved it from extinction.

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1. Keeley, J. E., Osmond, C. B. & Raven, J. A. *Nature* **310**, 694-695 (1984).
2. Moore, P. D. *Nature* **310**, 633 (1984).
3. Pigg, K. B. & Rothwell, G. W. *Bot. Gaz.* **144**, 132-142 (1983).
4. Masarati, D. L. & Thomas, B. A. *Phyta*, 155-162 (1982).

KEELEY *ET AL.* REPLY—We agree with Cockburn that, although the presence of CAM in astomatous *Stylites* does not support his theory on the stomatal origin of CAM, it does not necessarily falsify the theory either.

We also agree with Thomas that *Stylites* most reasonably should be included with *Isoetes*, a taxon dating back only to the Triassic. Lycophytes, however, extend back to the Devonian. Although it has been proposed that *Isoetes* represents a reduction from a tree-like lycopod, it is clear that herbaceous lycophytes have been present since the Carboniferous and form the longest unbroken time record of any group of vascular plants[1]. We do not believe that *Stylites* (*Isocetes*) *andicola* was the first vascular land plant, but it may represent a model system for how vascular plants invaded seasonally dry terrestrial environments.

The view of the morphological evolution of *Stylites* which Thomas presents agrees with the known fossil record of the Isoetales (see Thomas and Brack-Hanes[2]). It is also consistent with two arguments proposed by Raven[3] as to the physiological significance of morphological attributes in vascular plants, the first of which relates to the presence of xylem tissue in *Stylites*. The probable earliest function for xylem was that of water conduction rather than mechanical support. Transpiration does not necessarily imply the presence of stomata (compare with the extant *Polytrichum* and the Siluro-Devonian *Cooksonia*), but it does indicate that a phototroph obtains CO_2 from the air rather than the sediment. The second argument relates to the presence of intercellular air spaces which seem to be correlated with the presence, or occurrence at some stage in the plant's evolutionary history, of epidermal pores (Marchantiales) or stomata (sporophytes of vascular plants). These arguments suggest that *Stylites* was derived from transpiring ancestors that possessed stomata and derived CO_2 from the atmosphere rather than the sediment. However, Raven[3] noted that "the sequence of acquisition of H_2O-conducting and gas-exchange/regulation mechanisms revealed by the fossil record is generally in accord with teleological expectation, in that the H_2O-conducting system in the absence of the gas-exchange/regulation mechanism would be of more selective advantage than the reverse state of affairs". *Stylites* may represent such a survivor.

Thomas' conjecture about the evolution of *Stylites* is not consistent with our observations. Although it is possible that *Stylites* 'lost' stomata, the argument proposed by Thomas is not plausible; he points out that deep-water *Isoetes* (for example, *I. lacustris*) have totally lost stomata and that the lack of stomata in *Stylites* may derive from a previous aquatic ancestry. Although aquatic *Isoetes* species from deep water are astomatous, all such species so far tested (including *I. macrospora*, considered to be conspecific with *I. lacustris*[4]) will produce stomata on leaves initiated under 'artificial' aerial conditions (J.E.K., unpublished data).

The important feature of the astomatous habit of *Stylites* is the reliance on sediment carbon. Data on *I. triquetra* from the high-altitude paramo of the northern Andes (J.E.K., unpublished) and descriptions of the recently discovered *I. hopei* Croft from the topical alpine of New Guinea suggest that this syndrome, although rare, may be widespread globally in the Isoetaceae.

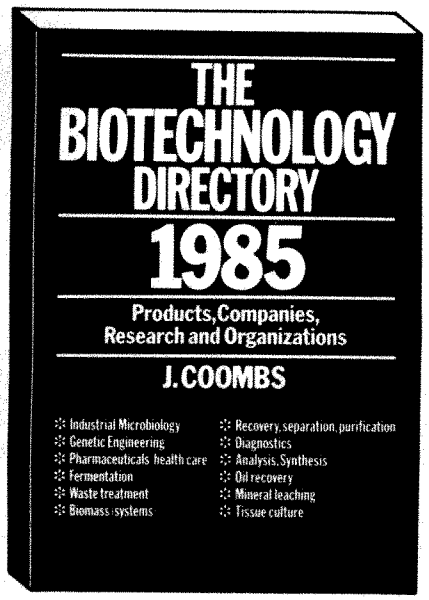
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1. Banks, H. P. *BioScience* **25**, 730-737 (1975).
2. Thomas, B. A. & Brack-Hanes, S. D. *Taxon* **33**, 247-255 (1984).
3. Raven, J. A. *Bot. J. Linn. Soc.* **88**, 105-126 (1984).
4. Kott, L. & Britton, D. M. *Can. J. Bot.* **61**, 3140-3163 (1983).

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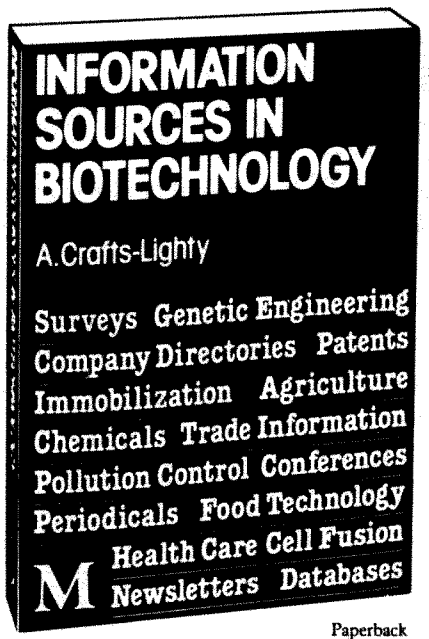
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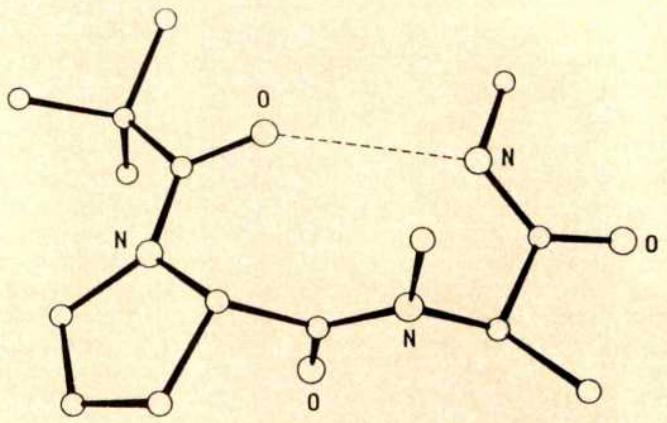
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The development of new drugs all too often involves the random screening of potential new compounds — a time-consuming and costly process. This book reviews how a more rational strategy to new drug formulation can be developed through the adoption of a multidisciplinary approach. Based on a symposium held in June 1982, three major aspects are discussed: the targets within the susceptible cell (including resistance); problems of the chemotherapy of infection (including cancer); and new approaches to chemotherapeutic strategy, which are of current interest and which may have value in the future.

Contents

Contributors; Preface; Antibiotics affecting DNA function — *S. Neidle*; Antimicrobial agents affecting membrane function — *S. Hammond*; Antibiotics affecting microbial cell walls — progress after 112 years — *S. Selwyn*; Antibiotics as probes of ribosomal structure and function — *E. Cundliffe*; Mechanisms of drug resistance — *J.T. Smith*; New strategies in the search for antiprotozoal drugs — *B.A. Newton*; Anthelmintic chemotherapy — *R.E. Howells*; Major problems in cancer chemotherapy — *K. Hellmann*; Chemotherapy of anaerobic bacteria — *A.T. Willis*; The strategy of drug development in hypoxic cell radiosensitization and chemosensitization — *I. Stratford*; Quantitative structure-activity relationships (QSARs) — principles and practice — *P. Wardman*; Three-dimensional aspects of molecules in drug design — *J. Tollenaere*; Targeting of drugs with liposomes — *G. Gregoriadis*; Index.

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Asdic to sonar

R.V. Jones

Seek and Strike: Sonar, Anti-Submarine Warfare and the Royal Navy 1914–54.
By Willem Hackmann.
Her Majesty's Stationery Office: 1984. Pp.487. £15.95.

THE detection and location of submarines in the sea has one factor in common with the analogous processes associated with finding aircraft in the air — the transmission of wave-borne energy. But while the air is a nearly uniform and transparent medium to electromagnetic radiation, and while an aircraft is a palpable discontinuity in that medium, the sea is effectively opaque over almost the entire range of the radio spectrum. It was therefore quickly realized that the detection of submarines would have to depend on acoustic waves — mechanical waves which need transducers for their generation and reception, a further contrast with radar, where radio waves are launched from and received by antennae which are simple extensions of electronic circuitry. As a result, once the problem of aircraft detection was formulated, the radar solution was brilliantly found and exploited to such an extent that in the decade 1935–1945 almost all the essential techniques had been developed.

No such exhilarating story can be told of submarine detection. Instead, it has provided one of the greatest challenges to the application of physics, where ground has been won only by prolonged effort. *Seek and Strike* records what was done in Britain between 1914, when the impact of the U-Boats was first felt, and 1954, when submarine warfare entered a new phase with the launching of the first nuclear submarine — the *USS Nautilus*.

The author, Willem Hackmann, is Assistant Curator of the Museum of the History of Science at Oxford, and he appropriately starts his account with the speculations of Aristotle and Pliny the Younger on the detection of underwater sound by fish. This has its analogy in the use of hydrophones to detect submarines by sound generated by their engines or hydrodynamically by their passage through water; this technique has been developed to detect submarines at ranges of hundreds of miles, but it is limited operationally both by the vagaries of transmission in a medium whose refractive index varies with salinity and temperature, and by a background of other noises generated not only by surface waves and ships, but also by such modest creatures as snapper shrimps.

While hydrophones can give general, and indeed impressive, warnings, ultrasonic echo detection — which gives range as well as bearing — is necessary to locate a submarine precisely enough for it to be attacked. Hackmann gives the credit for the original development of the method to Paul Langevin, but it may be worth mentioning that James Wilson in his recent biography suggests that some of it should also go to Rutherford.

The author has clearly done a great deal of painstaking work in pursuing the many aspects of a long and involved story, and in refining them into a readable and admirably documented account. Several points emerge from it. First, despite the fact that the Royal Navy appreciated as long ago as 1873 the importance of science when it founded the Royal Naval College at Greenwich to ensure that naval officers would study both science and mathematics, and despite the enthusiasm for science and engineering shown by Admiral John Fisher, few naval officers were prepared to take scientists fully into their confidence, even when faced with the U-Boat threat. Instead of letting scientists such as Rutherford experience the conditions of hunting U-Boats at sea for themselves, the chief naval liaison officer demurred, saying that all that the scientists need be told "was that the enemy submarines were in the sea and that means were required to detect their presence". Only after the advantages of close cooperation between scientists and serving officers had been demonstrated in air defence in the Second World War were the Services completely won over.

Another feature of the development of Asdic (or Sonar) was the continuous battle between long and short term interests: should greater priority be given to the development of a weapon for tomorrow or to research that might provide a much better weapon the day after tomorrow? The right answer depends on circumstances; but in naval weapons, such as Asdic or signalling, the answer was more often than not determined in favour of the short term, because appointments in the Navy have characteristically a two-year duration, and so captains of establishments such as The Signals School tended to favour programmes from which they might expect some concrete result within their term of office.

A further message is the danger of "overselling" a weapon, causing first an unjustified reliance on its performance in operations, and then a loss of confidence when its shortcomings become clear, as exemplified by Churchill's complaint to the First Sea Lord in 1941 about Asdic — ". . . the failure of our methods, about which so much was proclaimed by the Admiralty before the war". And yet another is the merit of trying a technique or system "the other way round", in this case the firing of the depth charges ahead over the bow of the hunting ship instead of dropping them over the stern, which led to an increase in success rate from about 7% to about 30% as a proportion of kills to attacks.

All these lessons, which have relevance far beyond submarine warfare alone, are to be found in the book. Alongside them are such morsels as the attempts of 1915 to train seagulls to hunt for U-Boat periscopes; the renaming of the quartz crystals required for piezoelectric transducers as "asdevite" to avoid disclosing their composition, and the recruitment of a firm of tombstone-makers to cut the crystals; and the fact that even a noisy submarine radiates no more energy than would light a 3-volt torch bulb.

Seek and Strike tells a story of successes tempered with failures in the sustained application of physics to one of the most crucial problems of warfare this century. It was well worth writing — and is well worth reading — both for its significance as history and for the lessons it exemplifies.

R.V. Jones is Emeritus Professor of Natural Philosophy at the University of Aberdeen and Chairman of the Defence Committee of the Centre for Policy Studies, London.



Pioneers of ultrasonic echo ranging; Paul Langevin is fourth from the left, wearing the bowler hat. The photograph was probably taken at Toulon in May 1918.

Breeding discontent

David Pearce

The Plutonium Business and the Spread of the Bomb.
By Walter C. Patterson.
Paladin: 1984. Pp.272. Pbk £2.95.

THERE are, says Walter Patterson, "nuclear people", "plutonium people" and "fast breeder people". Nuclear people believe in the civilian use of nuclear power for electricity. Plutonium people and fast breeder people believe in the original nuclear dream: the widespread use of plutonium — a by-product of burning uranium fuel — as a fuel for fast reactors which can breed more plutonium. Nuclear people need not be plutonium people but they share the same vision, that of a resource with enormous potential. If the resource is there they cannot understand why it should not be used. Yet resource use has costs as well as benefits; nothing comes free.

Patterson sets out to chart one of the costs of plutonium. The civilian nuclear power dream cannot be distinguished from plutonium as a weapons material, despite all the protestations by plutonium people to the contrary. This he does most successfully in an historical overview from Seaborg's identification of plutonium, through the Manhattan Project, to today's aggressive fast breeder programme in France and the limited triumphs and many failures elsewhere. The Super-Phénix is nearing completion in France at a cost of some $10,000 million. It is jointly owned by France, Italy, Germany, Britain, the Netherlands and Belgium. Patterson is quite clear how such a staggering cost is justified by advocates of the programme — it will provide military plutonium when other supplies have gone. We are all, it seems, helping to finance French nuclear weapons.

Patterson sees no hope in existing safeguards against the spread of military plutonium from civilian sources, whether in the International Atomic Energy Authority or the Non-Proliferation Treaty, the subject of so many ambiguous and confusing interpretations. For the truth is that no one knows collectively how much plutonium exists. One "best guess" for 1982 suggests some 175 tonnes of plutonium "in stock" in *civilian* sources in the non-communist world (though this appears not to account for high-purity plutonium from Magnox reactors which, if evidence to the Sizewell Inquiry is correct, actually has been diverted to US nuclear weapons). The annual production rate of plutonium is about 40 tonnes. The prospect of control thus seems remote.

In the same light it is easy to see why plutonium people are not concerned about the niceties of nuclear power economics. Any breeder reactor should be credited with the "value" of the plutonium it produces. By the same token, in the absence of a large breeder programme, all *conventional* nuclear power stations should be credited with the value of the plutonium in their spent fuel. That value lies in the benefit to world security from having nuclear weapons, a value many will find difficult to believe is less than a very large minus number indeed. But if this is the "real" economics of nuclear power why don't plutonium people admit it? The majority may not dispute the positive value of nuclear weapons. The answer may be that most plutonium people, just like most people, do not know the connection between civilian and military plutonium.

The alternative is that they *do* know the link but cannot see how stopping the civilian programme would help. After all, the weapons programme began with reactors dedicated to military plutonium. Electricity was incidental. Patterson offers no guidance on this point. For him it is enough that plutonium production is already out of control. With only a short time to go to the fortieth anniversary of Nagasaki we can only contemplate whether the lapse of time is the truest testament to man's ability to control his fearsome drive to self-destruction, or just an interlude between appalling but vastly different events. □

David Pearce is Professor of Economics at University College London.

Talk of Kyoto

F.S. Rosen.

Progress in Immunology V.
Edited by Y. Yamamura and T. Tada.
Academic: 1984. Pp.1,640. $110, £85.

IN August, Kyoto is inhospitably hot. Certainly Baedeker, if he ever had reached there, would have found it situated in an unhealthy valley, nonetheless worthy of being reached in slow stages from Edo by the great Tokaido so beautifully depicted in the wood-block prints of Hokusai and Hiroshige. Instead, in August 1983, some 4,000 immunologists were disgorged in haste from the bullet trains, arriving with frequent and clockwork regularity from Osaka and Tokyo, for their triennial self-congratulatory pow-wow.

Over 150 dense, compact summaries of all the symposium lectures have been published in this thick volume. It is a timely and state-of-the art overview of the whole field of immunology, in just over 1,600 pages of well-set, readable print. To read it from cover to cover is mind boggling, but certainly very informative. Summaries of the dozens of work-shops which took place during the congress are not included but that does not detract from the book; it might have resulted in overkill. Anthony Watkinson recently pointed out (*Nature* **312**, 201; 1984) that "the transformation of a transient three or four days of academic exchange into an enduring collection of reviews can be an invaluable contribution to learning". *Progress in Immunology V* measures up to just that.

On the fourth afternoon of the meeting, a spontaneously exuberant moment occurred when it became apparent that the T cell receptor had at long last been identified in Denver and Boston, in California and Texas. It was one of those rare times when an international congress is ignited by the tinder of a major discovery. Unfortunately, not all of the presentations at that session have been included. While dwelling on the omissions from this volume, it should be noted that there was almost nothing in the congress on immunodeficiency at a time when, among other things, AIDS is creating a stir and yielding information on lymphocytotropic retroviruses. There is also an omission of neuroimmunology at a time when immunology has provided interesting probes for the neurobiologist. And there is barely a nod in the direction of transplantation biology despite some good summaries of the current status of work on the major histocompatibility locus in mouse and man. Maybe all this will be fixed next time around.

Immunologists are returning to their roots with some highly productive and heuristic results. The articles by B.B. Bloom on leprosy, A. Capron on schistosomes and the Nussenzweigs on malaria are vivid demonstrations of how useful immunology can still be to its parent science. The emergence of synthetic vaccines also promises to burgeon into something usefully applicable, and current work in Israel and France on this subject is also well summarized.

The best subject coverage in the book concerns the molecular biology of the immunoglobulin and major histocompatibility genes. This field has moved very rapidly as can be gleaned from the outstanding summaries by D. Baltimore, L. Hood, T. Honjo and others. In contrast there are the still muddy fields of lymphokines, growth factor and other humors. They are desperately in need of being set right.

The high specific gravity of this tome is occasionally relieved by inspirational words, by D.W. Talmage, N.A. Mitchison and R.A. Good, that set the field in perspective. The opening reflections of B. Benacerraf epitomize the message of this meeting: "Nature rewards and yields its magic secrets to the most daringly imaginative. Hard work and dedication, while essential, are not enough". Time will tell if this challenge is to be met at the next congress in Toronto in 1986, and if that meeting too produces such a profusion of progress and excitement. □

F.S. Rosen is James L. Gamble Professor of Pediatrics at Harvard Medical School, and Head of the Immunology Division at the Children's Hospital, Boston.

An inside view

Sean C. Solomon

Planetary Interiors.
By William B. Hubbard.
Van Nostrand Reinhold: 1984. Pp.334. $42.50, £49.75.

THE United States planetary programme is midway through a five-year hiatus in major new observations from spacecraft missions, and an increasing number of American scientists are filling the gap by writing textbooks and monographs on various aspects of planetary science. Hubbard's offering treats the physics and chemistry of planetary interiors, covering all the planets (save Pluto) and most of the larger satellites viewed to date by spacecraft. The text incorporates material up to mid-1983, including results from the Voyager Saturn flyby and Pioneer Venus, but not the Soviet Venera 13-16 missions to Venus. A background in physics, chemistry, astronomy and geology at the level of a senior undergraduate or first-year graduate student is assumed.

The author has attempted a laudably comprehensive treatment of his subject. Discussion of the bulk composition and internal differentiation of each planet is cast in the framework of the composition of the Sun and the evolution of the solar nebula. Particularly thorough chapters are included on equations of state and planetary gravitational fields, both topics on which the author has made a number of original contributions. Accounts of the energy budgets of planetary interiors and the nature of planetary magnetic fields complete the foundation for the final half of the book, a thorough and quantitative inside tour through most of the principal objects of the Solar System.

Hubbard admits at the outset that "a single author has difficulty in writing a current, comprehensive book" on the planets. As if to prove the truth of this assertion, variations in the depth and evenness of coverage betray the background and research interests of the author. There is an implicit bias towards the outer planets, which first becomes apparent in the discussion on equations of state. The only silicate treated is "rock", defined as "that component of [nebular] condensate which is left after formation of an iron core". A chapter on the interior of the Earth is exposed as perfunctory with the opening apology that "this book is about the interiors of other planets"; worse, it contains misleading or incorrect statements about mantle convection, the nature of seismic discontinuities in the upper mantle and the evidence for a solid inner core.

There is a parallel bias towards an astrophysical, rather than a geological, approach to the planets. One telling statistic is that there is only a single photograph of a planetary or satellite surface (of the Discovery scarp on Mercury). The reader will find only cursory mention of cratering, volcanism, tectonics and lithospheric evolution on the solid planets and satellites. Much of the material on the terrestrial planets appears to have been taken either from the work of the author's colleagues or from a few selected review papers.

Perhaps these shortcomings are visible only from my own geocentric viewpoint. To an alien observer, the large outer planets would initially be the most interesting and an astrophysical approach to their study would be natural; indeed, our discovery and investigation of other planetary systems is, in fact, progressing in this fashion. At least from this cosmic perspective, *Planetary Interiors* is the most current and complete treatise available. □

Sean C. Solomon is Professor of Geophysics in the Department of Earth, Atmospheric, and Planetary Sciences at the Massachusetts Institute of Technology.

Ode to diversity

Lindon J. Eaves

In Praise of Difference: Genetics and Human Affairs.
By Albert Jacquard.
Columbia University Press: 1984. Pp.187. $20.

"THE essential nature of the Darwinian revolution was. . . the replacement of a metaphysical view of variation among organisms by a materialistic view". Thus Richard Lewontin (*The Genetic Basis of Evolutionary Change*, 1974, p.4) described the impact of biology on our understanding of life: an impact which has only been magnified by the ability, recently acquired, to extract and hold in our hand the molecular basis of life.

Against this background of the "scientific challenge" to traditional ideas, our acceptance of reproductive technology has already led to a radical adjustment in the norms of society with regard to the control of fertilization and the outcome of pregnancy. How far should we go down this road? In his preface Professor Jacquard writes:

> Human endeavor, based on a growing knowledge of both the inanimate and the living world, has become increasingly effective. Why not use this new power to achieve the most tempting objective of all: the improvement of the human species itself?

Popular ignorance of the main scientific ideas and facts involved is the first barrier to democratic decision-making, so Professor Jacquard begins by outlining the elementary reproductive and evolutionary processes as simply as possible and later discusses some of the mechanisms which can lead to the high level of polymorphism not predicted from classical theory in population genetics. His treatment of these issues is simple and lucid and will be readily comprehensible to the general reader. Having outlined the basic biological issues, Professor Jacquard focuses on subtleties which are sometimes ignored by advocates of a genetic basis to social policy and he tries to give a more balanced view of debatable ideas which are sometimes treated as "facts" in the popular mind. Among the problems he addresses are the "dysgenic" effects of medicine which, he argues, would take place extremely slowly even if they were not offset by such factors as genotype - environment interaction. His statement about consanguinity warrants some qualification. When writing of the origin of rules prohibiting consanguinity, he says (p.50), "the ill effects of consanguinity . . . are much too slight to be observed empirically". It must be presumed that he is thinking about cousin marriage, since the effects of closer consanguinity on mental retardation, for example, are well documented.

The book points, indirectly, to the separation of population genetics from quantitative genetics which has characterized much of the development of both disciplines. Professor Jacquard's strength is in population genetics. When he turns to quantitative genetics and psychometrics he does not do justice to the best of either subject. For example, he invests great effort in criticizing an additive model for individual differences without asking why such a model often fits the data remarkably well. And he criticizes the concept of general intelligence without addressing the more interesting question of why multiple abilities are correlated.

Bertrand Russell observed that moral considerations could only appear when the truth is known and should not be used to decide what the truth is. There are few moral propositions in the book from which the sensitive reader is likely to dissent. Man's unalienable right to "life, liberty and the pursuit of happiness" is maintained, whether it be given by God, history or DNA. In spite of the growth of knowledge, we do not have all the facts to manipulate the future of the species in the broadest sense. Is this so bad? Professor Jacquard suggests that it is not. The title of his book reflects his belief, expressed in the final chapter, that human diversity, guaranteed by the mechanisms of inheritance and natural selection, is a treasure which should not be sacrificed before any idol of "progress" or "uniformity". This principle extends as much to the fruits of man's intellect as it does to the species itself. The dissemination of scientific ideas, and their open discussion divorced from political influences, is the best insurance that science will remain a guardian of freedom rather than a slave to totalitarianism. □

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Practice of science

Russell Moseley

Philosophy and Sociology of Science: An Introduction.
By Stewart Richards.
Basil Blackwell/Schocken Books: 1984. Pp.210. Hbk £17.50, $20; pbk £5.95.
An Introduction to Science Studies: The Philosophical and Social Aspects of Science and Technology.
By John Ziman.
Cambridge University Press: 1984. Pp.203. £12.95, $22.95.
The Experience of Science: An Interdisciplinary Approach.
By Martin Goldstein and Inge Goldstein.
Plenum: 1984. Pp.400. $22.50, £21.38.

THE more dogmatic features of an undergraduate education in science (almost as bad as orthodox theology according to Thomas Kuhn) are well known and are enshrined each year in thousands of examination questions which require candidates to demonstrate their competence in solving puzzles within well-established frameworks. Those examining conventions which today we take for granted have not always been so closely observed. Cambridge science students in the 1850s were invited to consider whether there was "a shadow of proof from the ethnographical and physical history of man that any one of his oldest varieties was derived from a quadruminous progenitor" and were urged to state their own opinions "without hesitation" should they differ from those of the examiner. But by and large undergraduate science students have not been encouraged to question either the status of the knowledge acquired in their apprenticeship or, indeed, the nature of the scientific enterprise more generally. Not surprisingly then, studies which seek to encourage such questioning have met with a mixed reception.



Visitors to the cave paintings of Lascaux in France, shortly after the cave's rediscovery in 1940. The picture is reproduced from the new paperback edition of The Creative Explosion, *by John Pfeiffer, published by Cornell University Press. For review see* Nature **302**, *764 (1983).*

To reduce the possibility of outright rejection it becomes necessary to establish the legitimacy of "science studies" and to convince students of the need to view them as an integral part of a scientific training rather than something which derives from a nineteenth-century belief in the virtues of a liberal education. In this respect students are more likely to take notice if these matters are addressed by *bona fide* scientists, and all three of the books reviewed here share this advantage. Credibility in students' eyes may be further enhanced by claims for the utility of science studies, and each book contains a justification for such teaching over and above "metascience" (as Ziman calls it) for its own sake. Thus Richards begins with a recollection of the value to his later career of an undergraduate course in the history and philosophy of science, while Ziman sees "a possible guide to action in scientific research, in industrial management, in political administration, and in public affairs". Once credibility has been established a major problem remaining for the would-be teacher of science studies is the choice of material — there is no agreed core curriculum here but a spectrum extending from the philosophy of science to the economics of technological innovation. This is a difficulty which confronts any author writing a book on science studies, and the problem of providing material and a structure which will be sufficiently flexible to find use in a broad range of courses has no easy solution. Since all three books make claims to be suitable undergraduate texts, their success in this respect deserves careful consideration.

An additional obstacle to be overcome stems from the constantly growing specialist literature generated by those historians, philosophers, sociologists, economists and political scientists who contribute to the science studies area. Both Richards and Ziman are acutely aware of this — their titles make it clear that we are dealing with "an introduction" — and both authors offer their work as a guide to an interpretation of the wider literature and as a means of presenting themes which they identify as being of particular importance. For a reviewer this poses a problem. To engage in a discussion of the authors' interpretation of debates in the philosophy of science, the sociology of science, science policy and so on, would be an undertaking which would result in another (introductory) text. Instead it seems preferable to focus on the appropriateness of the particular guided tours we are being offered in the light of their stated purpose.

The routes taken by both Ziman and Richards are, at first glance, very similar, starting with a broad look at selected features of the philosophy of science, moving on to the sociology of science and science policy, bringing us finally to more general cultural matters. Both tours finish rather abruptly, with neither guide attempting any recapitulation of those features which were of special interest — presumably they are taken to be self-evident if the tourist has been paying attention. Despite the apparent similarities there are some significant differences. On the whole the Ziman route is the more stimulating, revealing some unexpected views, striking out on paths only recently discovered and generally traversing the terrain at a faster pace. The Richards party will certainly get their money's worth, but will miss some of the newer landmarks, will avoid some of the higher slopes, and generally travel more sedately.

Ziman is noticeably the stronger in his discussions of recent work in the sociology of science and is more successful in linking his themes throughout the book. Richards more clearly divides his work into "Methods and Philosophies of Science" and "Interactions of Science and Society". The least satisfactory part of both books is that dealing with the organization of research and development and technological innovation — at this point the tourist may find his or her attention wandering (to be fair, neither author sets out to treat the broad area of science, technology and society comprehensively, but questions concerning the economics of research and development and technical change are a part of science studies, and one for which students often show considerable interest). Similarly, the history of science is allowed little more than a token appearance. Students using either of these books will need to be reminded that the Ziman and Richards routes are not the only ways of seeing the landscape and that others have been mapped out.

The Goldsteins offer an alternative approach, and the intended use of their book as an interdisciplinary introduction to science means that metascientific questions are viewed from a different perspective. There is, in addition, a welcome historical dimension introduced through case-studies, those commodities which are always so eagerly sought by teachers of science studies. As with the other two books it would be possible to direct students to specific sections rather than treat the text as a whole. Indeed, some selection of material is probably inevitable since few science studies teachers have sufficient time at their disposal to permit more than a sampling of the range of topics introduced in the three books, let alone persuade students to read into the chosen areas as suggested. Stimulating as these guided tours may be, the problem remains of translating them into the one or two hour a week teaching session typically allowed for such studies in the science curriculum. □

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nature

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Etiquette for arms control

Politicians in the United States and the Soviet Union have a serious responsibility towards the arms control negotiations at Geneva — they must keep more quiet than is their habit.

THE best news from Geneva last week is that there was very little of it. Too many earlier conferences on arms control have been ruined by the recklessness of senior politicians on one side or the other in taking it into their heads to utter public statements about the course of the negotiations. Sometimes they have even advertised their "negotiating position" before letting the other side know privately what they have in mind. When people spill the beans like this, they often believe they are acting sensibly. The motive in going public is usually that such a step will strengthen the hands of negotiators operating behind closed doors, if only by reducing their freedom for manoeuvre. The difficulty is that successful negotiations demand a degree of flexibility, not to be mistaken for a readiness to compromise on issues of principle. In negotiations like that now begun at Geneva, the slow and inevitable leakage there will be about the conduct of the argument is immaterial. What will be damaging are statements made in public outside the negotiations which are intended to influence their course, or which declare that the outcome (whatever it may be) will be of no importance. Let us hope that there will now be a moratorium on public speaking.

Keeping quiet will be harder for the United States than for the Soviet Union. Constitutional pluralism can be a diplomatic embarrassment. But the United States is also caught up in several nasty almost domestic squabbles about strategic weapons, from Australia and New Zealand to Belgium (where the government's decision to let cruise missiles be installed has lent strength to the anti-nuclear movement). It will not help that the debate continues in the US Congress about the administration's authority to build another clutch of MX missiles. On past form, it is almost certain that some administration official will be tempted to go imprudently far, and in public. It is ironical that the dangers of the MX debate would be lessened if the United States were only to recognize that, even on military grounds, the MX is a mistake. It is too big and clumsy, and carries so many warheads that its deployment will entail putting too many eggs in too few baskets. (Each missile that fails on take-off will presumably have to be replaced by another, kept waiting in the wings.)

The Soviet Union, where plurality is not conspicuously a problem, is more strongly based. Mr Mikhail Gorbachev's accession may even help, by allowing subtle changes of policy seem less like concessions to pressure from outside. For what it is worth, the Soviet Union has, in any case, recently abated its earlier fierce complaint about the star wars issue. Condemnation of the idea persists, of course, and Soviet spokesmen keep saying there can be no agreement at Geneva without an agreement on the "demilitarization of space". That is legitimate enough, although the point has been made so often that it should now be repeated only in private (at Geneva). But the demand, loudly heard six months ago, that there should also be a moratorium on the testing of components of a space-based strategic defence has been quietly dropped. (A moratorium on the testing of anti-satellite weapons is a different issue, and would be valuable in its own right as well as an aid to a successful negotiation.) Temptations to speak out unwisely will nevertheless, no doubt, arise.

Meanwhile, both sides at Geneva are saying that the negotiations will take time, but that they could in the end succeed. That they take a longish view is a welcome sign of realism. But it would have been more encouraging if the two sides had settled for a more empirical way of working, one that did not require that success should hang on a solution of all the world's problems at one stroke. For most people, a few modest steps towards a more comprehensive agreement would be preferable to the promise of an all-embracing agreement that could be snatched away by the simplest failure during the next few years. Conversely, a few modest agreements now — why not settle for ratifying the unratified treaties on the threshold test-ban and peaceful nuclear explosions? — would give the negotiators time to tilt at the big windmills.

The most obvious weakness of the arrangements on which the two sides at Geneva have agreed is that success hangs critically on success in each of three parallel sets of negotiations, on missiles of long and intermediate range and on star wars. The linkage between the first two issues is natural, given the strategic interest of Western Europe and other regions of the world, but star wars is logically separate, at least now. (Further ahead, it the United States had a working missile defence, the Soviet Union would be forced to build more missiles or to do something else.) The haunting difficulty is that if star wars had never been conceived, the missile negotiations would now be just as difficult as those that aborted at Geneva at the end of 1983. Is it not asking a lot, to hope to succeed now in an all-embracing negotiation when there was only failure to report in the simpler circumstances of eighteen months ago? After so much disappointment, why do the arms control diplomats allow themselves to be frustrated by the over-ambitious goals they set themselves?

To the errors of garrulity and over-expectation that repeatedly catch out the practitioners in this esoteric trade must be added a third — poverty of imagination. When arms control negotiations between the superpowers began, nearly thirty years ago, it was customary for the practitioners to try to visualize how things must seem to their opposite numbers, on the other side of the table. The view "from over the hill" was the standard phrase. That exercise seems to have lapsed. And both sides are to blame.

The Soviet Union, for example, has been inscrutably insensitive to the West's perception of its development in the strategic field. The large increase in the numbers of SS20 missiles (which have intermediate range) deployed in Western parts of the territory during the past ten years is shrugged off as if it were irrelevant to the strategic balance in central Europe. More recently, the phased-array radar that has popped up in Siberia, apparently in violation of the anti-ballistic missile treaty, went unexplained for more than a year and was then passed off as a component of a space-tracking network, as if there is nothing more to be said (or asked) about this installation. No doubt developments of a political character are even more unsettling of opinion over the hill. As things are, it must be assumed that the Soviet Union has given up trying to anticipate the impressions its actions cause.

The same, unfortunately, seems to be true of the United States. Nobody seems to have invited President Ronald Reagan to consider how the frequent claim that star wars (or authority for MX missile building) has forced the Soviet Union "back to the negotiating table" must seem inside the Soviet Union, habituated to the notion that the world is against it and now economically insecure as well. The claim, often heard, that the Soviet Union

has no business to react so angrily to star wars because its potential for making a first strike from the West feasible is a strictly academic matter, given that the United States would never do such a thing, must sound disingenuous in places such as Omsk and Novosibirsk. Even the assumption, commonly voiced in the United States, that the superior high technology of the United States (in the civilian field at least) will always ensure success in an arms race, may sound aggressive in the East. So should not Western politicians, like those elsewhere, learn not merely to hold their tongues for the duration of the Geneva negotiations, but also spend a little of the time they will save trying to see things from the other fellow's point of view? □

Hearts in the wrong place

The unauthorized use of an artificial heart in Arizona was mistaken humanity.

NATURAL sympathies are with the Arizona surgeon who two weeks ago commandeered an unapproved artificial heart in an effort, ultimately futile as it happened, to keep his patient alive while a replacement human heart could be found. The surgeon, Dr Jack Copeland, has been cast in the traditional American role of the rugged individualist defying authority to do what is right. The Food and Drug Administration (FDA) has obligingly filled in with the part of the pointy-headed bureaucrat by mumbling about technical violations of regulations.

The facts of the case are these: a 33-year-old man who had had two heart attacks and a poor chance of living much longer was given a new human heart in what is becoming an increasingly routine operation. The new heart, however, stopped beating the next day. While Dr Copeland began the search for a replacement, the patient was placed on a heart–lung machine. As the hours passed — and a patient can be kept on the heart–lung machine only a few hours before his red blood cells begin to be damaged — Dr Copeland decided the only hope for his patient was the temporary implantation of a mechanical heart.

FDA had authorized only one surgeon — Dr William DeVries of the Humana Heart Institute in Kentucky — to perform that procedure, and only with the Jarvik-7 heart, which was developed at the University of Utah. For reasons that are still unclear, Dr Copeland called St Luke's Hospital in Phoenix, and asked surgeons to bring a mechanical heart under development there. That heart, developed by a dentist, had not been submitted to FDA for approval. It had never been used in a human. Its longest test had been in a calf, for 12 hours. (The Jarvik heart was tested for months in a calf before the first clinical test.) A Jarvik-7 heart was flown in as well, but was not used; some reports say the University of Utah decided against participating in the unapproved surgery.

The Phoenix heart kept Dr Copeland's patient alive for 121 hours; a new human heart was then transplanted but by then lung damage had taken its toll and the strain on the new heart was too much. The patient died, three days after the ordeal began.

Dr Copeland and his surgical team say, simply, that there are higher laws than FDA when a patient's life is at stake. Yet the very point of FDA's regulatory role is to prevent the sort of haphazard and unplanned experiments on humans that in fact took place here. Informed consent — which was obtained from the patient's relatives — is only one necessary element in assuring the ethical practice of experimental medicine. In a crisis, what relative is going to say no to a doctor, especially one so obviously sincere as Dr Copeland in wanting to do the best for his patient? It may be a cruel thing to say, but the end result of what happened in Arizona is that a human being was used as a guinea pig. The FDA rules, hardly extreme, are designed to assure a minimum degree of safety and likely benefit before humans become the experimental subjects.

The irony of the entire episode is that, with better planning, what was attempted at Arizona is really the way that artificial hearts ought to be put to use now: as a stop-gap during heart-transplant surgery. After the Arizona episode, FDA did in fact approve yet another artificial heart — this one developed in Pennsylvania — for just this purpose. Heart transplants can now offer real hope for otherwise hopeless patients. They have also succeeded in many cases in allowing patients to resume near-normal, active lives. The same simply cannot be said for the "permanent" implantation of artificial hearts at this primitive stage of development. An implant that must be tethered to a huge external air compressor (apart from posing a constant risk of infection at the point where the tubes enter the patient) exacts a devastating psychological toll.

The argument, of course, is that the early heart transplant operations could have been criticized on similar grounds — that they were grandstand medicine, more stunts than attempts to improve the patients' well-being — but that what was learned in these many early attempts paved the way for today's successes. The analogy is not exact, however. The medical spectaculars at the Humana Heart Institute (which has permission from FDA to go ahead with more "permanent" artificial heart implantations) are turning out to be gruesome endurance tests. No matter how much is learned about surgical technique, proper medication and the like from these operations, the technology of the heart itself is simply too primitive to justify the goal of permanent implantation. They are attempting to fine-tune a procedure that is questionable even if it worked perfectly.

Admittedly, clinical performance data need to be collected hand-in-hand with efforts to improve the technology. But limiting the artificial heart to temporary use seems a reasonable compromise to achieve this goal without turning humans into guinea pigs. □

Sustaining Africa

The UN now has money with which to feed Africa. How should it be spent?

THE United Nations has surprised itself by collecting (in promises) more than twice the $1,000 million it had asked for so as to reinvigorate African agriculture. No doubt part of the success stems from this season's appalling famine in Ethiopia, worse than last season's by a long way if only because it was more elaborately covered by television cameras. Unfortunately, it is far from easy to tell how such large resources could be spent wisely and quickly enough to make much difference within the lifetime of those who have worked to recruit the funds. The temptation will be to spend the money on the classical agricultural projects of development — irrigation dams and drainage schemes. But the needs are social and even political.

Ironically, most industrialized countries honour their farmers and are indeed over-generous towards them, but developing countries, where the need for food is greater, tend to worry instead about the prices that urban people (and voters) pay for the food they eat. The result is price control that pauperizes the farming population, driving them to the cities to look for welfare instead of growing food. On many occasions, matters have been made worse by spectacular mismanagement, as of Ghana's cocoa crop, once the foundation of the country's relative prosperity. In Ethiopia, part of the cause of this year's famine must be the hostility of the government (and, fair play, of the farmers) towards the middlemen who traditionally bought grain from farms and sold it on to cities; nobody remembered that the middlemen were the distribution system, which collapsed when the intermediaries were abolished. There are also, of course, climatic and agricultural problems of great severity.

The most urgent need, and the best use of the UN money, is for a technical infrastructure to support peasant agriculture, Africa's equivalent of the extension services of Europe and North America (but also in place in India before the green revolution). The second need is for better plant breeding, done in Africa (but not necessarily by Africans.) The third is that African governments should recognize that the whole country will go hungry if farmers are driven to starvation by low prices. There might even be some change left over for a few dams. □

Genetic engineering patents

Contest in prospect over interferon rights

Washington

BATTLE lines are now being drawn up in what may be the first major contest for patent rights over a commercially significant product of genetic engineering.

The product, human leukocyte (alpha) interferon, has shown potential in clinical trials against some cancers and viral diseases, including the common cold. Last year Biogen NV of Cambridge, Massachusetts, and Geneva, Switzerland, announced it had received a European product patent for alpha interferons produced by recombinant DNA techniques; the company expects shortly to be issued with a US patent covering the same claims. Now, however, Hoffman-LaRoche Inc. of Nutley, New Jersey, has announced that it has received a US patent covering "all highly pure human leukocyte interferons no matter how they are made". John Saxe, chief patent counsel for Roche, says the company is "not aware of any highly pure leukocyte interferon product that does not fall within the issued claims". Schering-Plough Corporation, which produces Biogen's interferon (trademarked Intron) under licence, immediately challenged Saxe's claim, denying that Intron fell within the scope of Roche's patent.

The Roche patent is based on the 1970s work of Sidney Pestka and Menachem Rubinstein, in which they isolated and purified alpha interferons from human cells. LaRoche expects to receive a further US patent covering its own recombinant alpha interferon, Roferon-A, which was developed in collaboration with Genentech Inc. of San Francisco. The patent already issued to Roche defines interferon by its anticytopathic activity as assayed by two standard cell lines but does not include sequence data; Biogen's European patent, in contrast, does specify the product by sequence. A further complication is that Biogen's patent, which was filed before that of Genentech/Roche, strictly refers to an interferon precursor molecule, not the mature molecule, according to Genentech.

Alpha interferon is undergoing clinical trials at several centres in the United States. Diseases in which it has shown potential include Kaposi's sarcoma (often associated with acquired immune deficiency syndrome), multiple myeloma, malignant melanoma and hairy cell leukaemia. Both Schering-Plough and Roche have submitted marketing applications to the Food and Drug Administration for their respective products, which differ by a single amino-acid residue. Schering's Intron is approved for use in two indications in Ireland and the Philippines.

The courts have yet to test patent law as applied to genetic engineering products, and many small companies are seriously concerned that their product options might be constrained by broad product patents. Both Schering-Plough and Roche, as major corporations, have the resources to endure a court battle should one become necessary: Peter Feinstein of Biogen says he is confident that Schering will be able to prevent Roche from marketing Roferon-A. But industry analysts point out that large pharmaceutical corporations tend to avoid court cases wherever possible, and some sort of compromise may yet be negotiated.

Whatever the resolution of the present dispute, it is unlikely to serve as a model for future cases; with each product patent issued, the extent of "prior art" increases and future patents become harder to defend. One possible pointer to the future, however, is the recent US patent granted to Cetus Corporation covering what it calls muteins of interleukin-2, a lymphokine with anticancer and antiviral potential. The muteins are specific modifications of the natural protein: the Cetus mutein is claimed to be more stable than natural interleukin-2. Just as important, however, Cetus is confident that its mutein will lie outside the scope of other interleukin-2 patents.

Tim Beardsley

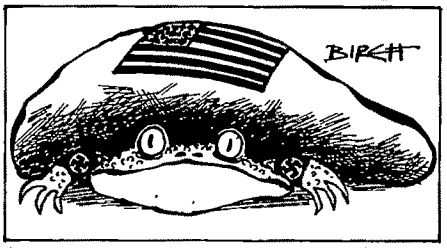
Military manoeuvres

Scientists' Nazi past laundered

Washington

US ARMY intelligence officers deliberately altered the dossiers of German scientists brought to the United States at the end of the Second World War to conceal their Nazi party activities and, in some cases, even their indictments for war crimes. Government documents released for the first time leave little doubt that the Army's intention was to circumvent President Truman's order that no "active supporter of Nazism or militarism" be allowed into the country under the programme of recruiting German "specialists".

Among those whose dossiers were altered was Wernher von Braun, the rocket scientist who later assumed a leading role



in the US space programme. Von Braun's original dossier labelled him a "potential security threat".

The Army also attempted to recruit four scientists who were later indicted at Nuremberg for war crimes. One was sentenced to 20 years' imprisonment for participating in experiments in which concentration camp inmates were forced to drink sea water.

The documents, which are the first to show a deliberate cover-up by the Army, were obtained by a reporter for the *Bulletin of the Atomic Scientists* under the Freedom of Information Act. A full account will appear in the April issue of the *Bulletin*.

Under President Truman's order, the State Department had to pass on scientists recruited by the Army. In early 1947, the State Department refused visas for several scientists labelled in security reports as "ardent Nazis". The director of the recruitment programme, Bosquet Wev, then complained to the Army's intelligence director that "the best interests of the United States have been subjugated to the efforts expended in 'beating a dead Nazi horse'". Wev said "the most positive and drastic action" was needed. The most revealing document came later that year: Wev again wrote to Army intelligence, and enclosed dossiers of 14 scientists, including von Braun: "...security reports recently forwarded from your headquarters classify 14 specialists as potential or actual threats to the security of the United States...there is very little possibility that the State and Justice Departments will agree to immigrate any specialist who has been classified as a...security threat...It is requested that the cases of the specialists listed in paragraph one be reviewed and that new security reports be submitted where such action is deemed appropriate in view of the information submitted in this letter." All were subsequently changed.

Other dossiers — such as those of the scientists indicted at Nuremberg — appear to have been "clean" from the start. The dossier of Arthur Rudolph, the NASA scientist who left the United States last year to avoid being deported for his role in the persecution of slave labourers at the V-2 rocket plant that he managed during the war, made no mention of evidence from the Nuremberg trials implicating Rudolph nor of his having joined the Nazi party in 1931.

All told, 765 scientists and technicians were recruited under the programme. The documents confirm that a major concern of the US officials who ran the programme was not letting the German scientists fall into Soviet hands. Wev wrote in one memorandum: "The return of these scientists to Germany would present a far greater security threat to the United States than their retention in this country".

Stephen Budiansky

Anthropology

Genetic links for scattered Jews

Rehovot

PRELIMINARY studies using DNA sequences as a new and sophisticated tool for genetic analysis tend to support the conclusion drawn from earlier investigations that the Jews, even after being scattered around the world for two millennia, remain — to a significant degree — genetically distinctive.

This view, propounded for many years by Professor Batsheva Bonné-Tamir from the Department of Human Genetics at Tel Aviv University's Sackler School of Medicine, has met with opposition from some other scientists who use different statistical methods and from those who feel that any attempt to suggest the existence of a specific Jewish group is to be rejected as racist doctrine. Professor Bonné-Tamir is obviously not a proponent of such a doctrine, nor does she suggest that the Jews are better or worse for having a common genetic heritage. She simply points to the evidence that it exists.

Among the genetic markers used in the past by Professor Bonné-Tamir are blood types, enzymes, serum proteins and histocompatibility antigens (see *American Journal of Human Genetics* **34**, 50; 1982). The new data, which have been submitted for publication, come from the most direct genetic measure — that of DNA sequences, in this case of short stretches of mitochondrial DNA. But whatever the criterion, she and Professor Samuel Karlin of Stanford University in the United States find that Jews from Iraq, for example, have far more in common from a genetic viewpoint with Jews from Poland than either group has with the non-Jews among whom they have lived for centuries. This is also true of immigrants to Israel from such diverse areas as Germany and the Soviet Union on the one hand and Libya on the other.

It has become possible to make more revealing comparisons between Jews and non-Jews in recent years because of the increasing number of genetic studies on both.

There are, it seems, a few exceptions to the general picture of relative Jewish distinctiveness. Jews from Yemen, often popularly regarded as the Jewish group that looks most "biblical", are a case in point. "They have a genetic make-up", Bonné-Tamir reports, "that is characteristic of the Saudi-Arabian peninsula and are probably the descendants of indigenous tribes which converted to Judaism in the fourth and fifth centuries."

A similar situation prevails in regard to Cochin Jews and the local Kerala Indians, communities that are genetically linked to one another but have little in common, from a genetic viewpoint, with other Oriental-Jewish groups or with non-Jewish elements in southwest Asia. This fits in well, Professor Bonné-Tamir points out, with the theory proposed by Professor Avinoam Adam of Everyman's University (Israel's equivalent of the UK Open University), who has written: "in some areas and during some specific periods, the Jewish religion expanded much faster than Jewish genes".

Most timely of all, in view of the present wave of immigration from Ethiopia, are her findings about the Jews from that country. Various theories exist as to their origins, with members of the community itself tending to argue that they are descendants of the tribe of Dan (one of the original 12 tribes of the Bible) and/or of ancient Jewish communities in Upper Egypt that moved south. Yet after studying representatives of a hundred immigrant families from Ethiopia, Professor Bonné-Tamir comes to the conclusion that genetically they most resemble the Amhara tribe, and to a lesser extent the Billen and Tigre tribes, of Ethiopia. But she says "there is no denying the fact that the Yemenite, Cochin and Ethiopian Jews have long displayed strong emotional bonds with Judaism and rushed to join their co-religionists in Israel as soon as they had the opportunity to do so."

Nechemia Meyers

Australian technology

OECD begs leave to differ

Canberra

AS soon as the Organization for Economic Cooperation and Development (OECD) draft report on the state of Australian science and technology was made available last month, it became obvious that many of the recommendations for improvement were exactly the opposite of those in operation at present or likely in the near future. The examiners asserted that it was essential that the science and technology portfolios be combined and represented at Cabinet level by a strong minister. An Australian federal election was held between the first draft of the report and its submission to the Australian authorities, the upshot of which was a portfolio reshuffle, splitting Mr Barry Jones's Department of Science and Technology, with politically important policy sections being absorbed by Senator John Button's Department of Industry and Commerce (see *Nature* 5 January, p.4). Mr Jones's remaining Department of Science has in the meantime been casting about for new vistas in science without technology, a condition described by the examiners as "perverse", because, in their view, the topics are so completely interwined. The government's action also puts paid to the examiners' recommendation that the Australian Science and Technology Council (ASTEC) should report to a "strong science minister" rather than supply policy advice directly to the Prime Minister, which has been its brief since 1976.

The difficulty is the notion of a single strong minister for science and technology. Senator Button certainly fills the bill for his now-extended Department of Industry, Technology and Commerce, but part of the reason for the political downgrading of Mr Jones's portfolio was his unwillingness to linger in smoke-filled rooms where the deals are done. It is difficult for citizens of nations where science and technology are not (yet) an issue to appreciate the job of advocacy that Mr Jones has done in Australia, and the void that existed before him. The examiners noted that many of the depositions submitted to them in the course of the study bore witness to the discussions he had sparked in 1983-84, but something else shone through as well: a sense that science and technology were somehow external to national life and that the transition from research to design and sales of a product seemed to involve the collision of mutually uncomprehending cultures.

Part of this they attributed to the education system and recommended (*inter alia*) that research in universities and other tertiary institutions be strengthened, especially by the purchase of modern equipment, both propositions unlikely in the short run, given the Treasury's present parsimonious mood. The examiners supported some tactics — such as manpower planning — that are unlikely to find favour, and suggested that Australia should not follow blindly the example of other countries in developing "key technologies" such as information technology and biotechnology, but instead examine its own latent strengths on a sectoral basis, suggesting pharmaceuticals as a possible new industry. Whereas in the past Australia has used bounties and tariffs to shore up industry rather than the system of fiscal incentives common in other OECD countries which encourages business enterprise to classify its activities as research and development for tax purposes, the examiners recommended that tax-based schemes be instituted in Australia, a strategy the government has already begun and may expand.

Although preceded by an OECD *rapporteur* a year ago, the examiners themselves spent only one week in Australia. Inevitably, their report must be coloured by views expressed in the submissions made to them. They seem awake, nonetheless, to the pressures that have been brought to bear on OECD referees in the past, noting that familiar arguments about the economic benefits of investment in intellectual and technological infrastructure may be needed for the benefit of the Commonwealth Department of Finance. A final consultation and draft are due later this year.

Jeffrey Sellar

Soviet policy

Gorbachev on science

SOVIET scientists, and, indeed, Soviet society at large, must pay greater attention to the challenge of modern technology, according to the new General Secretary of the Communist Party of the Soviet Union, Mikhail Gorbachev. Addressing the Central Committee last December, in place of the ailing Konstantin Chernenko, Gorbachev noted that although in many enterprises and scientific institutions research and development problems are resolved "up to the standards of the world's top achievements", many "collectives" are still satisfied with repeating what has been outstripped in world practice.

Paramount importance, Gorbachev told the Central Committee, must be attached to the strategy for developing scientific, technical and production potential, and, in particular, to "fundamentally new and really revolutionary scientific and technical solutions" which will increase labour productivity many times over.

This has been standard party policy for some time; what is, perhaps, significant is Gorbachev's emphasis on the idea that scientific and technological progress can be achieved only by raising the "cultural and technical standard" of the working class and peasantry, and "a radical improvement in the training and perfecting of society's main productive force". This, he said, will entail a major restructuring of the "cadre training system" at all levels of education from primary school up to university. This work is already under way, he noted, but "must acquire a large-scale character".

Educational reform, in particular the changes introduced in September 1984, is apparently a subject in which Gorbachev takes a special interest. One of his last major engagements before his elevation to the general secretaryship was to chair a meeting of the Commission on the Reform of General and Vocational Education. The 1984 reforms stress "practical labour training, and vocational orientation" for all school-children, and the appropriate production ministries are expected to help provide necessary facilities. The February meeting of the commission, however, found that the All-Union Ministry of Light Industry and a number of light industry ministries in the union republics have in many cases contented themselves with drawing up "plans for measures and instructions". Local government officials and education committees, likewise, have been doing little to implement the reform.

This slackness, the commission decided, will not do. Within the next two to three years, it warned, all school-children must be properly involved in socially useful and productive labour, and at the same time, all secondary-school pupils must be computer-literate. **Vera Rich**

Bendectin/Debendox

Drug not guilty, says court

Washington

A FEDERAL jury last week decided that there is no evidence to link Bendectin, an antinausea drug used in pregnancy, and birth defects. The court, in Cincinnati, Ohio, dismissed a case brought by 1,100 women across the country, representing two-thirds of outstanding US claims against Merrel Dow pharmaceuticals, the maker of the drug.

A spokesman for the company said the decision established that a product liability case could be successfully defended in the United States and indicated that Merrel-Dow will now be in no mood to propose no-liability settlements in the remaining cases. Earlier, the company had offered $120 million in settlement of all outstanding and future claims.

Bendectin (known in the United Kingdom as Debendox) was available as a prescription drug in the United States from 1956 to 1983, when it was withdrawn by the company because of high insurance premiums resulting from the accumulating claims and the bad publicity. Merrel Dow says that no fewer than 27 epidemiological studies have failed to turn up any evidence that the drug causes birth defects. The active ingredients are doxylamine (an antihistamine) and vitamin B6. Before 1976, Bendectin also contained dicyclamine, which was removed because of insufficient evidence that it added to efficacy.

The Cincinnati ruling is the third courtroom vindication for Merrel Dow. A case in Florida in 1981 was decided in the company's favour; in 1983 a jury in Washington DC awarded one plaintiff $750,000 damages but was overruled by the trial judge. The publicity made Bendectin a potential bonanza for tort lawyers, some of whom started to advertise for parents of children with birth defects who might have been affected by Bendectin.

Recognizing that the mass of litigation threatened to clog the legal system, the trial judge in the most recent case took the innovative step of holding a common issues trial to decide questions relevant to all the 1,100 plaintiffs. In an attempt to avoid having to face future litigation on Bendectin, Merrel Dow offered $120 million as a class settlement to cover all future claims. The offer was accepted by the majority of attorneys in the case but was challenged by some dissidents; the class settlement was then disallowed by an appeal court on a technicality, necessitating the trial that ended last week. **Tim Beardsley**

Soviet Union

More nuclear research

THE Dubna Joint Nuclear Research Institute in the Soviet Union is to expand its area of research considerably during the coming five-year plan (1986–1990), its director, Academician Nikolai N. Bogolyubov, announced last month. Addressing a meeting of government plenipotentiaries of the 11 member states of the institute (the 10 Comecon partners plus North Korea), Bogolyubov noted that during the next five-year plan "great attention" would be paid to identifying applied work, using the results of fundamental research in related spheres of science and technology, in particular medicine, geology, biology, metallurgy and ecology.

Applicability of fundamental work is a keystone of Soviet science policy, and in a formal keynote speech need not have anything other than a purely ritual significance. Bogolyubov, moreover, has a background in fundamental mathematics and physics and has just been awarded the Lomonosov medal, the highest honour of the Soviet Academy of Sciences, for his theoretical work. Nevertheless, at a time when Comecon research plans in all fields are being closely coordinated, the commitment of Dubna to finding new applications for its research probably represents a genuine statement of intent, especially for the less prosperous member countries.

In his speech, Bogolyubov put considerable emphasis on the "powerful experimental base" that Dubna has acquired in recent years, including the "unique" pulsed neutron fast reactor which came into operation last year, and the plans for the construction of a nucleotron — a superconducting accelerator with the aid of which, he claimed, it will be possible to accelerate nuclei of virtually all the elements in the Mendeleev table, right up to uranium. Dubna's latest achievement, announced shortly before the February summit, also lies in the theoretical field: the creation of element 108. According to the official announcements, in the spring of 1984, in work on the new U-400 cyclotron at Dubna, 44 cases of the decay of three isotopes of element 108 were recorded.

In spite of the "experimental base" praised by Academician Bogolyubov, however, not all is well at Dubna. The central computer centre, in particular, seems to be inadequate, judging from the stress placed on the need to enlarge its capacity. The "instrument centres" of the various laboratories, too, need to be expanded.

● The Soviet Union, of course, has other atomic research institutes. Foremost among these is the Kyrchatov Institute of Atomic Energy in Moscow which, says Academician Evgenii Velikhov, has a considerable lead over the West in fusion research. **Vera Rich**

CERN

How to pay British share

BRITISH Foreign Office subscriptions to international bodies are effectively protected against exchange rate fluctuations by the Treasury — so why should not the international subscriptions of the Science and Engineering Council (SERC) be similarly protected? Two Oxford high-energy physicists, Professor Don Perkins and Dr John Mulvey, have put this question to their local Member of Parliament, Tory John Patten, who has promised to raise the matter with Sir Keith Joseph (Secretary of State for Education and Science). Sir Keith is still awaiting news from the Kendrew committee on Britain's future role in high-energy physics, so the physicists' intervention is opportune. Friction between high-energy physicists and others supported by SERC, which pays the annual subscription to CERN (the European Organisation for Nuclear Research), is exacerbated by the fluctuating cost of the subscription in pounds sterling against what is now a constant subscription in Swiss francs, and any realistic way of handling the fluctuations would at least ease tempers.

According to Perkins and Mulvey, the issue is this. Member states of CERN pay their subscriptions in Swiss francs according to their "net national income" (NNI) averaged over three years. The proportions are determined every three years using the most recent statistics of the United Nations Statistical Office. But these statistics can be three years old, with the result that actual payments can be six years out of line with real current NNI. "For example, the present shares were decided in 1983, using data for 1979-81. They will be in force for 1984-86."

Nevertheless, in the long run, exchange rate fluctuations are automatically corrected, as NNIs are calculated in dollars. So if sterling falls the British NNI falls with it. But the delay in calculations causes trouble. Recently, the sterling cost of the CERN subscriptions has risen sharply from £23 million in 1981 to £35 million in 1984 because sterling, and hence NNI, was strong against the dollar in 1979-81 but has fallen nearly twofold since.

According to Perkins' and Mulvey's calculations, the net overspend (out-turn minus estimate) by SERC on the CERN subscription over the eleven years starting 1971-72 was only £4.75 million (just over one per cent of the total). From year to year, however, the overspend has been as high as £4.5 million (1976-77) and as low as minus £8.7 million (1980-81) — a profit for SERC.

"Exchange rate and relative NNI changes are quite outside the control of anyone", write Perkins and Mulvey, "and the application of 'cash limits' (inclusive of the international subscriptions) to the already desperately strained SERC budget does not encourage economy. It only subjects the scientific programmes to arbitrary jolts which have very damaging, and wasteful, effects."

Thus they recommend:

- That subscriptions to international research organizations (such as CERN) should remain part of the budget of the appropriate scientific policy-making body (for example SERC). "Then the trend of the budget... can be decided in the right scientific context."
- That the delay in the calculation of relative contributions be shortened, "though this may not be welcomed by countries with more stable currencies".
- That SERC could pay foreseen subscriptions in sterling, while the Treasury corrects for changes in the exchange rate and NNI by adding or subtracting the appropriate sums. "This separates the functions of making scientific policy (SERC) and solving the technical problems (Treasury).... Experience shows that the Treasury (and SERC) would not lose (or gain) over a number of years."

Moreover, Perkins and Mulvey claim, "there can be no objection in principle to such a mechanism since cash limits are not applied to the international payments made by the Foreign and Commonwealth Office".

Here, however, there appears to be some uncertainty. According to a Treasury spokesman, Foreign and Commonwealth Office (FCO) international subscriptions (to the United Nations, for example) are indeed not cash limited, which means that the Treasury will bail out FCO if there are political changes in those subscriptions during a financial year, but on currency fluctuations, FCO must look for "offsetting savings", according to the Treasury. However, unlike SERC, FCO has a long-standing "arrangement" with the Treasury by which the fluctuations are discussed as they occur. **Robert Walgate**

NSF deputy at last

DR John H. Moore, an economist at the Hoover Institution in Stanford, California, has been nominated by President Reagan to be deputy director of the National Science Foundation (NSF), a post that has been vacant for two years.

Although Dr Moore has an undergraduate degree in chemical engineering and worked as an industrial research chemist for four years, his PhD is in economics, a subject to which he has devoted the bulk of his professional career.

Moore has been a member of the National Science Board, NSF's formal governing body, for the past two years.

The Hoover Institution is a generally conservative think-tank associated with Stanford University. □

Archaeopteryx

Fraudulent feathers?

SCIENTISTS at the British Museum (Natural History) met on Monday to launch their response to the mounting publicity surrounding the claim by Lee Spetner in Rehovot and a group including Fred Hoyle and Chandra Wickramasinghe from University College Cardiff, that the feather impressions on their specimen of the famous fossil bird-like reptile *Archaeopteryx* may be a fake.

The controversial claim has been raised by an editorial and an article accompanying new photographs of this specimen published by the group in the *British Journal of Photography* two weeks ago. The crux of the claim is that many of the feather impressions occur on patches of material that are much finer-grained than the underlying rock and in parts the material looks like "flattened blobs of chewing gum". The possibility is that this represents some sort of cement that was applied to the fossil after discovery, on which feather impressions were made.

To elevate the argument from rhetoric and refutation, the scientists at the museum decided at their meeting to carry out two tests on the fossil specifically to address the possible existence of a cement layer. They will first take a section of material from the edge of the specimen for standard microscopic sedimentational analysis to detect any differences in particle size and the existence of a boundary between a surface layer and underlying material. They will then use electron microprobe analysis to compare X-ray spectra emitted by surface and underlying material.

The team is not, however, optimistic that these tests will provide clear-cut evidence and is already aware that a cement made from underlying rock might not be detected by these analyses. Although ready to carry out these tests immediately, the team feels it may be best if the areas selected for analysis are chosen by Hoyle's group.

The decision to allow samples of material to be taken from this extremely important fossil results from the conviction, voiced by Dr Alan Chong of the Department of the Palaeontology at the museum, that the specimen is genuine. He would seem to have considerable support from the existence of five other specimens assigned to *Archaeopteryx* from the Upper Jurassic. All have associated feather impressions but were discovered at different times and at different sites. Indeed, the incomplete Tyler specimen discovered in 1855 was first described as a pterodactyl, and it was not until 1972 that John Ostrom noted the feather impressions and described it as *Archaeopteryx*. If the feather impressions are the work of a single faker, he must have been remarkably fleet of foot and very prescient. **Nigel Williams**

Genetic diseases

Problems of prenatal testing

Washington

NEW techniques for prenatal diagnosis of genetic diseases are sharpening the debate in the United States over when the Food and Drug Administration (FDA) should approve for commercial use test kits that might lead a woman to prefer an abortion to the possibility of bearing a handicapped child. Methods based on analysis of fetal DNA promise to increase greatly the number of diseases for which prenatal diagnosis is possible, but the anti-abortion lobby is firmly opposed to any test used in a "search and destroy" mission. And the anti-abortion lobby cannot be dismissed as part of the lunatic fringe: President Reagan has made clear his opposition to abortion under any circumstances.

Prenatal testing for some easy-to-detect conditions such as Down's syndrome and Tay-Sachs disease has been carried out for several years. Although in some instances a good argument can be made that tests have increased, rather than decreased, births of healthy but potentially affected children, the strength of anti-abortion sentiment suggests that the arguments will have to be fought anew as each new test becomes available. Furthermore, the spread of chorionic villus testing, which allows fetal cells to be obtained earlier in pregnancy than by amniocentesis, could make easier those abortion decisions based on tests for inherited conditions: the National Right to Life group is already challenging use of the technique.

FDA spokesmen are wary of speculating about the regulatory passage of future commercial kits, but it is clear that any company wishing to market a new prenatal diagnostic will have to go to unusual lengths to provide data on false positive rates before approval can be expected; a false positive result could lead to the unnecessary abortion of a healthy fetus. Industry spokesmen hope that FDA will restrict itself to questions of safety and efficacy, disregarding the thorny risk/benefit issue, with its attendant questions about the quality of life of a handicapped child. But some recent FDA decisions — on putative cancer therapies, for example — suggest that FDA does indeed consider it part of its job to scrutinize possible benefits as well as safety.

In 1983, after seven years of foot-dragging, FDA eventually approved unrestricted use of test kits to detect elevated concentrations of α-fetoprotein (AFP) in amniotic fluid or serum, indicative of neural tube defects such as spina bifida. That decision reversed FDA's earlier position, in which it had sought to specify the testing protocol and interpretation of results for AFP kits. Although it is pointed out in defence of FDA's delay that there are good reasons to be wary of uncritical use of AFP tests, few tests would claim to be 100 per cent efficient — in any case, numbers matter little to those whose opposition to abortion is based on religious grounds; it remains to be seen whether future tests will experience similar delays.

Many prenatal diagnostic tests, such as those for sickle cell anaemia and phenylketonuria, are now being performed in university medical centre research programmes, approved by institutional review boards but not by FDA for unrestricted use. Commercial reference laboratories, in contrast, use only FDA-approved reagents. The limits of FDA's authority to regulate experimental tests are "somewhat cloudy", according to an official in FDA's compliance division: although there is no question that an experimental test can be used for evaluation in conjunction with an established technique, or if it presents no significant risk, established tests are sometimes lacking, and abortion decisions are being taken on the basis of experimental tests. There is at least one published case of an abortion resulting from incorrect experimental diagnosis of a haemoglobinopathy.

For some of the genetic conditions that can now potentially be diagnosed by analysis of linked fetal DNA markers, such as retinitis pigmentosa, Duchenne muscular dystrophy and Huntington's disease, testing of relatives is necessary (but not always sufficient) to arrive at probabilistic estimates that a given fetus is affected by an inherited disorder. As long as this sort of specialized knowledge is required for testing, work will be restricted to experimental medical centres and so remain effectively beyond FDA's purview, even though such testing is offered as a commercial service.

But the goal of much current research is to produce probes that will identify a mutant gene directly, or, even better, the gene product. Recently, for example, a direct gene probe for prenatal detection of phenylketonuria has been obtained. When simpler tests become available error rates will fall, and commercial kits will be feasible. One of the greatest hopes is for a test for cystic fibrosis, the commonest lethal genetic disease in the United States. The gene responsible for the disease has not yet been identified, but development of a test is "at an advanced stage", according to Integrated Genetics Inc. of Massachusetts. Although the eventual FDA approval for AFP test kits is hailed as a precedent for future tests, the industry knows that it will have to work harder in future to satisfy FDA demands. **Tim Beardsley**

Geology

New Chinese institute

A NEW Chinese research institute, the Xian Laboratory of Loess and Quaternary Geology (XLLQG), has been set up in Xian, Shaanxi Province. The laboratory is an independent unit, attached to Academia Sinica, and is engaged in the study of the characteristics and formation processes of loess and other Quaternary sediments in China. On the basis of the geological and biological records of the Quaternary period, the workers at the institute will try to reconstruct the history of the environmental changes of the Quaternary, and to understand the effect of human activities on future developments. This reconstruction of the pattern of environmental change is the single most important task facing Quaternary investigators, and those involved with XLLQG will be well placed to make significant contributions.

Xian is in the middle reaches of the Yellow River with the great Loess Plateau and widespread deserts to the north and west. To the east, Xian faces the North China Plain, and to the south, across the Qin Ling mountain, is the Red Basin of Sechuan Province with temperate and subtropical landscapes. The continuous loess-palaeosol series which began to be deposited 2.4 million years ago (see *Nature* **300**, 431; 1982) provides one of the best available indicators of Quaternary climatic changes, and contains abundant traces of early humans and Quaternary vertebrates.

Several research projects are already under way; these include investigations on the composition, physical characteristics and micromorphology of loesses and palaeosols and their relation to environmental changes and engineering construction; processes and mechanisms of recent dust falls and loess sedimentation and the relation of loess accumulation and loess erosion; the evolutionary history of human fossils on the Loess Plateau and the migration of fauna and flora during the Quaternary; loess-palaeosol sequences and their meaning to palaeoclimate since 2.4 Myr BP, and the sequence pattern and its development in northern China; and the recent climatic change (over about the past 2,000 years) and its relevance to human activities on the Loess Plateau and in northern China.

The first director of the new institute is Liu Tungsheng, with An Zhisheng as deputy director. Liu says he expects XLLQG to be a laboratory of international character. It is hoped that scientific and technical cooperation between China and other countries will not be limited to the exchange of research workers, but will also include joint ventures. This idea is fully supported by Academia Sinica and the new institute should provide a chance for investigators from all over the world to work in one of the really classic loess regions. **Ian Smalley**

Radiation data wanted

SIR — In 1975, the International Commission on Radiological Protection (ICRP) published its Publication 23, the Report of the Task Group on Reference Man. Publication 23 is about 15 years out of date, because the references cited end around 1970. At its 1984 meeting, ICRP approved the formation of a new task group to revise and update this publication, with completion expected in about three years.

The revision will include more emphasis on variations due to age, sex and other individual differences in anatomical and physiological data and in the gross and elemental composition of tissues. The emphasis in Publication 23 is on data for radiation workers, and the new emphasis reflects the fact that radiation doses to the whole population are of increasing interest to ICRP and national bodies interested in radiation protection.

The purpose of this letter is to solicit information from users of ICRP Publication 23, incuding workers in the medical and biological sciences outside radiation protection who have used the document. In order that the revised document may be accurate and complete, it is important that we get advice from as many users as possible. The present task group would appreciate hearing about any errors, omissions of important information or shortcomings in Publication 23 or of relevant new data not included.

Please write to the task group chairman at the address below.

C.R. RICHMOND
(Chairman)
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Creationism

SIR — As our organization, the Association for the Protection of Evolution (APE), has been accused of misrepresentation and an ethical lapse by Professor E.H. Andrews in his response (*Nature* **312**, 396; 1984) to our report of the Biblical Creation Society (BCS) open day (*Nature* **311**, 703; 1984), we feel obliged to reply. First, if Professor Andrews claims to be a theist, why is he engaged on a project involving natural theological argument and the acquisition of empirical evidence from the natural world for the existence and putative activities of a God — a paradigmatically deistic position implicitly shared by all creationists whether they understand the meaning of the word or not? Second, Mr Malcolm Bowden (for it was he) has on several occasions castigated BCS for its dangerous backsliding tendencies towards the position of theistic evolutionism. Third, Andrews waxes indignant at the suggestion that he has criticized US "scientific" creationism; we need only quote from his seminal article in the BCS "journal", *Biblical Creation* (vol.5, no.16) to show that he has been criticizing US creationism for some time. In this, the first "Biblical Creation Lecture", he writes "the American branch of creationist movement (*sic*) has therefore attempted to develop a scientific creationism that has no religious content". He goes on to say that such creationism involves "a number of very real dangers and problems", is "a-Christian" and "devoid of biblical content". His lecture (which we have on tape) merely amplified his previously published criticisms.

Chris Darnborough was introduced as doing research on genetic engineering and evinced an adequate knowledge of modern genetics, unlike all the other creationists we have so far encountered. APE made a theologically acceptable *in pectoris* decision to elevate Darnborough to the honorary position of BCS's top geneticist in default of any other candidates. The title of his lecture was, after all, "Genes created but EVOLVING" and he did have to apologize to his audience in advance in case they mistook the bulk of his lecture as putting the evolutionist view.

As for APE's "ethical lapse", in reporting Dr Gower, he did indeed state his belief that faults in the perfection of the "design" of the human olfactory organ are due to the effects of the fall of Adam and/or the work of the Devil. Whether he believes this enthusiastically or unenthusiastically is a matter of judgement. Our opinion was the latter. In any event, APE explicitly informed the organizers of the conference that it would be reporting their deliberations for the scientific press.

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SIR — The article "Louisiana law thrown out" (24 January, p.257), described the US court system's rejection of a law that would have required teachers to include "creation-science" along with "evolution-science". The law defined "creation-science" to be "the scientific evidence for creation, and inferences from those scientific evidences". This law required that students be given crucial data, even if they support a theory contrary to the beliefs of the teacher. Teachers who believe the creation account of origins would be required to include the evidence for evolution, just as the proponents of evolution would be required to discuss the evidence for creation. The rejection of this law encourages censorship of data about origins.

The inherent religious nature of *all* versions of origins must not be minimized. It is true that the creation account of origins is consistent with some "theistic religious views". The theory of evolution is consistent with the tenets of non-theistic religions as well as the tenets of the "main-line" and "liberal" churches. All views of origins support someone's religion: there is no religiously neutral theory of origins.

Students have a right to know that the lack of intermediate fossils discredits Darwin's theory of evolution. Students should also be informed that the modern belief in molecular evolution is held in spite of the fact that real observations require the rejection of spontaneous generation. The causes behind the shift in opinion away from creation in the 1850s should be reviewed and discussed with students. The citizens of Louisiana wanted to ensure that crucial data, such as the gaps in the fossils and the Law of Biogenesis, will be part of public school curricula.

The students must be able freely to form their opinions without the fear that critical data have been withheld. It is sad that one federal court and the American Civil Liberties Union seem to disagree.

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Missing mass

SIR — I would like to make only a point of general scientific method in reply to your reviewer who found "glaring errors and omissions" in my book *The Hidden Universe* (*Nature* 24 January, p.329).

The book attempts to explain the subject of "missing mass" to the layman. Surely the significant point about this subject is the small but increasing body of observational evidence that has now brought sceptical astronomers round to believe in this phenomenon. The point is *not* the plethora of mythical particles, or other agencies, which can all too easily be advanced to fill up extragalactic space. Precisely because we know so little about the phenomenon, it is of course at present hard to test out the presence there of axions, heavenly chariots or discarded bandwagons. That being so, economy of effort alone directs our attention first to those candidates that are either known to exist, such as neutrinos, or at least lead to some testable predictions. Supersymmetry and GUT [Grand Unified Theory] particles may be appealing to some, while reminding others of epicycles. For the moment, GUTs have fluffed their first proton-decay test, and the fact that they contain 21 readjustable free parameters could be held to lessen rather than add to their appeal in the context of missing mass.

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BIOSYSTEMS UPDATE

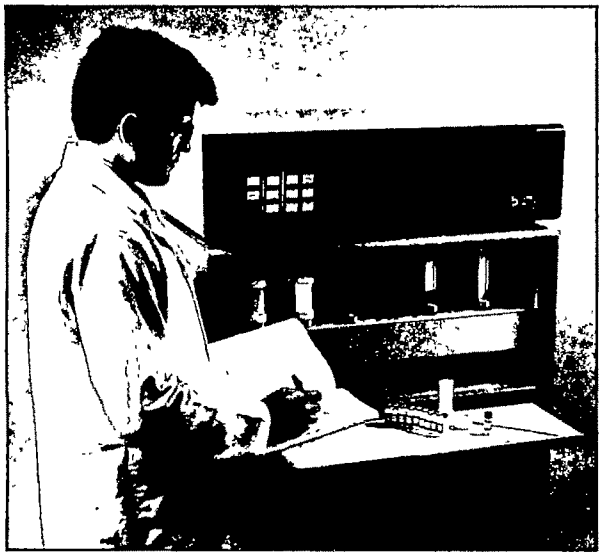
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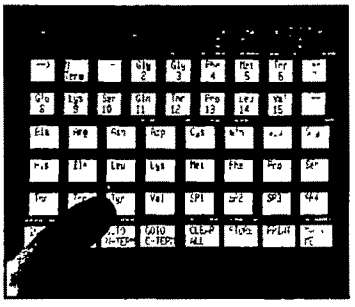
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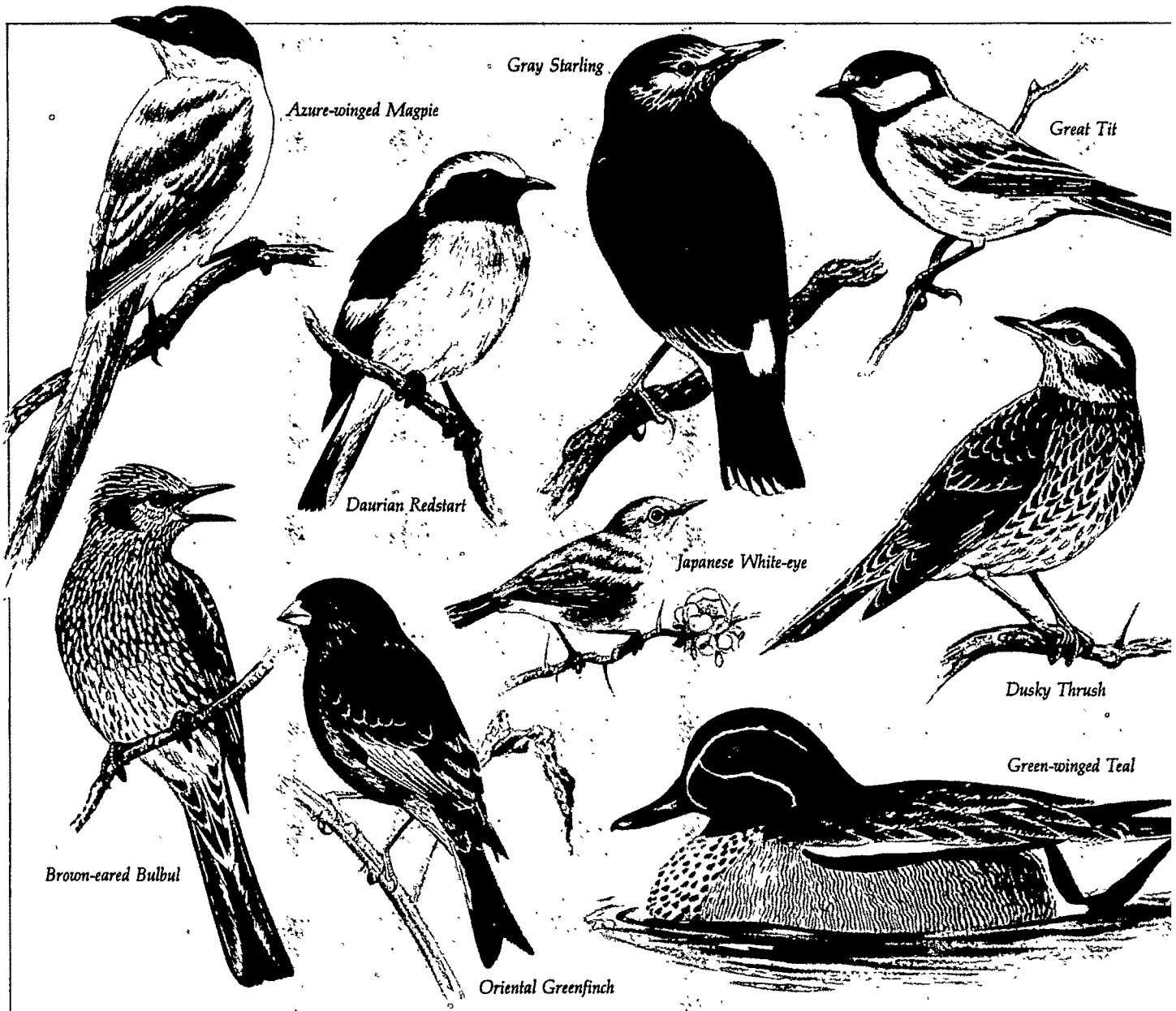
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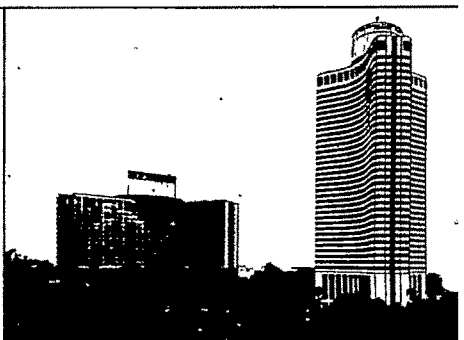
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Robots and images galore

Japan's massive science and technology fair, Tsukuba '85, opens this week in Tsukuba Science City. It is well worth a visit.

AT the end of the movie showing in Toshiba's pavilion at Japan's Expo '85, a young Japanese boy turns to a white-coated scientist and symbolically hands him a baseball. A robot with expressive eyes who has guided him around a research lab looks on, cooing electronically. The boy has decided to put aside his dreams of becoming a baseball star and become a scientist. The overwhelming message coming from the Japanese pavilions at the international exposition — on the theme "Dwellings and surroundings — science and technology for man at home" — is "Love science and technology".

The message comes in various forms:

"The affluent life of the twenty-first century begins amid the harmony of life and science", whisper the loudspeakers in Mitsubishi's "Wonderful world, beautiful people" pavilion as viewers ride cars on a trip beyond time and space.

"As management systems advance, accuracy and safety will increase, this will allow everyone in the community to enjoy life", says the commentary in Hitachi's rotating Interface Theatre.

"Technology can be fun", say the slogans in the Fujitsu pavilion.

And the epilogue in the government's history pavilion is "While each part acts independently, the whole functions with a beautiful order; just as the relation between the body and the cell so the relationship between society and scientific technology will grow". Alongside the message a sculpture of waving disks on tall, flexible poles demonstrates overall harmony in independent movement; surrounding video monitors show aerial views of *masu geemu* (mass games) of people on a playground — only when viewed from afar can the overall order in apparently chaotic movement be discerned; ever-changing flower-like patterns emerge. "This is the future harmony between man and technology", explains a guide clad in a silver uniform cut into geometric shapes.

The Japanese faith (officially, at least) in the benefits that technology will bring seems untroubled by the doubts entertained in other nations; indeed, perhaps it is just this naive faith the Expo sells that will produce the miracles it predicts.

At any rate the amount of organization and effort that has gone into the Expo is truly spectacular, as is the resultant 250-acre site covered with futuristic pavilions from the Japanese government, 28 Japanese corporations, 47 foreign countries and 37 international organizations. Spectacular too is the number of people expected to attend. Recent surveys suggest that the original guess of 20 million visitors during the six months of the Expo is on the low side, more like 26 million people are likely to attend — more than one-fifth of the entire Japanese nation. What better way to accustom people to the high-technology future that lies ahead? That is, if all 26 million people can get there — huge traffic jams at the preview suggested that it is not going to be easy.

The Expo has not been cheap to set up. Some 210,000 million yen ($840 million) has been spent, with one-third of it coming from private industry and the rest from the government. The official theme of the Expo, on "Dwellings and surroundings", seems not to have been taken that seriously. At the very beginning, it had been intended to hold an Expo simply on the theme of science and technology, but it had been pointed out (by the controlling body for international expositions in Paris) that this would virtually bar all but the advanced nations from participation. Thus the adoption of a more specific theme; it was thought (by a somewhat dubious bit of logic) that the less advanced nations could display technology appropriate to their particular "dwellings and surroundings" and the emphasis on high technology lessened. Indeed, that and very generous financial terms offered to the developing countries by the Expo organizers has ensured greater Third World participation than at any previous Expo. But for the Japanese contributors to the Expo — the two government pavilions and the 28 corporate pavilions that dominate the Expo and take up around 70 per cent of its space — it is still fundamentally an exposition of science and technology pure and simple. A walk around two-thirds of the pavilions revealed a model of only one dwelling (a solar house), no contribution from construction companies, and rather little in the way of new items for the home; the emphasis is rather on the boundless (and often rather abstract) advantages of high technology presented with all the razzmatazz of a fairground.

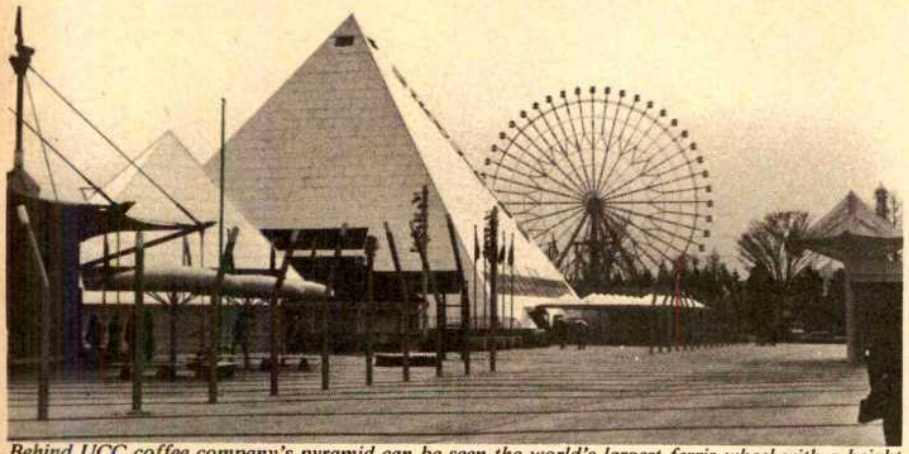
In building the pavilions, and the corporations have lashed out around 2,000 million yen ($8 million) on each of their own, Japan's top architects have been put to work. Or rather the top well-established architects have. Despite looking to the future, Japan's young architects have not had a look in; it is Kurokawa Kisho (who designed the Japan exhibition held at the Royal Academy in London a couple of years ago) and Maki Fumihiko who lead the pack. The result has been a plethora of pythagorean solids: cones, spheres and pyramids galore dot the site. The IBM (Japan) building manages cleverly to combine a cone, a sphere and a square; the Toshiba building's ground plan is of a square, semicircle and triangle. Clearly the days when geodesic domes were inextricably linked to visions of the future are over; the only big domes are the two of the Midori-kan's biotechnology pavilion, linked together to represent cell-fusion technology. That is not to say that curves have been entirely rejected; the government Expo Plaza building looks like a giant white butterfly come to rest — a pair of outstretched scalloped wings roof an open space for the performance of festival shows. The US pavilion, by Maki, evokes a graceful series of billowing sails and the Electric Power pavilion consists of dozens of white tent-like structures with sharply-pointed peaks pulled out by wires running to the top of a tall, slim tower.



The Tsukuba Expo abounds in the world's biggest smallest and fastest. This is the Sony Jumbotron, the world's largest television. Some 25 metres high and 40 metres wide, beside projecting futuristic images it will be used for a world news service.

Tsukuba Expo '85

THIS feature is by *Nature*'s Tokyo Correspondent, Alun Anderson, except where indicated. Japanese names are written with the surname first. The International Exposition, Tsukuba, is to run from 17 March to 16 September. The Expo site can be reached from central Tokyo in 2 hours.



Behind UCC coffee company's pyramid can be seen the world's largest ferris wheel with a height of 85 metres. Each gondola can carry eight people. There are worries that it may be struck by lightning, as the plain surrounding Tsukuba is notorious for mid-summer thunderstorms. The organizers assure doubters that it is absolutely safe and that the odd thunderbolt will only add to the excitement.

Traditional Japanese touches are provided too, again by Kurokawa. A long line of simple buildings with walls of cream ceramic squares set in black steel frames recall the sliding screens of traditional Japanese houses. A few pavilions have aimed at the downright exotic: Shueisha has a massive mock stone building covered in primitive carvings and exhibits a mummy from the British Museum for its theme of "Historical proof of Man"; and Daei's "The home of the poet" building is a low, stepped pyramid with its entrance hall underground.

Like expositions elsewhere, almost everything on the site is to be torn down when it is over (the area will become an industrial park), so the building of fantastic and ephemeral forms has been encouraged. It is impossible not to be impressed by a walk around the site, not only by the originality of the buildings themselves but by the sense of space with which they have been laid out.

Strangely, although the government has repeatedly said the whole exposition will present a "vision of the future", in actual fact no coherent picture of what future life may be like emerges. There are some individual exhibits — like that of NTT's Information Network System — that deal with bits and pieces of the future. But mostly on display are images — on giant screens, in 3-D, on high-definition televisions — and robots.

In the Fuyo robot theatre there are fifty self-propelled robots of twenty different kinds (but mostly of a very cute kind that only children will be able to tolerate), a talking robot roams the main open space (and switched into English while talking to this correspondent and followed him shouting "I want to go to England"), a robot plays the organ and reads music in the theme pavilion, Matsushita's robot draws portraits after a ten-second look at a face, Hitachi's assembly robots get out of the factory and spend their time sculpting ice blocks, Toshiba's robots spin traditional tops and can send them along tightropes to one another, Fujitsu has the world's largest humanoid robot. More than science and technology itself, maybe it is robots that you must learn to love!

But why, in Japan of all places, has it been found necessary to go to all this trouble to inculcate a love of science and technology in the citizenry? Clearly, both industry and government have already decided that Japan's chief hope for continuing rapid growth and prosperity lies with high technology — innumerable government reports and exhortations have already driven the point home and the key products for the new era are on display at the Expo — intelligent robots equipped with sensors, electronic imaging equipment, machine translation systems, high-definition television.

But strangely, when seen against Japan's volume of high-technology exports, the people of Japan have perhaps had less contact with the new technology than elsewhere, and science and technology are still very much seen as the domains of "experts". Take some of Tokyo's most highly automated big offices for example; they may boast that they lead the "OA" revolution in Japan but the fact is that workers spend rather little time at electronic keyboards and rather a lot of time in old-fashioned face-to-face group discussions. Often companies are not themselves using on a large scale the technology they are selling abroad. Again, small computers are very much more widely diffused in schools and homes in the United Kingdom than they are in Japan. An exhibit in the British pavilion claims that "Young girls and boys in Britain are now learning the language of new technology as naturally as their mother tongue"; given the number of small computers available in Britain it may well be true. The same could not be said of Japan, even though Britain's computers are doubtless full of Japanese microchips.

Also the pace of Japan's industrialization has left some groups out of touch. The rapid post-war shift of population to the cities has left behind a population in the countryside with a very different level of contact with high-tech; older people, too, feel more threatened by technology and will have had a much clearer experience of its ill-effects. Those who lived through the 1950s and 1960s, when nothing was allowed to stand in the way of high growth, will have clearer memories of Japan as the pollution capital of the world: Minimata disease, photochemical smog, arsenic accidentally introduced into milk, Itai-Itai disease....

In one sense, then, the Expo aims to strengthen a national consensus that the only way to move ahead is with high technology, to prove that science is not something "dry and stiff" but is something to enable people "to live in happiness". The Expo too is a festival celebrating just how much Japan has achieved already and how much technology is now part of daily life.

Apart from a little revision of history in one government pavilion, there is no evidence of nationalism or of a desire to boast of Japan's very remarkable achievements. But Japanese visitors to the Expo must feel an immense sense of pride in the wonders that are on display there. Perhaps it will bolster their sense of optimism for the future too.

The Expo also serves several subsidiary purposes, the principal one being to put Tsukuba Science City, on the fringe of which Expo stands, on the map. For all its



Nature's Tokyo correspondent as seen by Fuyo's robot.

46 laboratories and institutes, its two universities and its 34,000 scientists, and the million million yen that has been spent on it, it still has not captured the imagination of Japan's citizens (see *Nature* **305**, 378; 1983). Perhaps the Expo will make it seem a brighter, more exciting place and help attract the population it is still lacking.

The style in which propaganda for the twenty-first century has been put over is very typically Japanese, even though the Japanese themselves may not be aware of

it. Two separate but entwined influences can be seen at work. First, there are the two trends in Japanese art (and life): towards extreme understatement, the ultra-refined, and the use of absence and emptiness to suggest presence; and towards the noisy, the colourful, the boisterous and the vulgar.

The first world is that of traditional Japanese gardens where a few rocks and some carefully raked gravel suggest a universe, of the exact placing of a few flowers in *ikebana* (flower arrangement), of the slow and subtle gestures of the masked actor in a Noh play, and of careful understatement in speech. This side of Japanese aesthetics is the better known in the West. But the other extreme also exists of wild and drunken festivals, of colourful Kabuki plays, and of grotesque and garish Edo-era paintings of actors and prostitutes and demons. In origin, the two cultures belong respectively to the samurai that ruled Japan throughout its feudal period, and to the common people, particularly those of Osak and Edo — what is now downtown Tokyo.

Much of Japan's commercial television seems to spring from the latter source: there always seem to be two or three people shouting at once, lights are flashing, and girls are shrieking in an endless party from which everyone with a trace of introversion has been excluded. But a walk around Tokyo reveals that the opposite trend has by no means left Japan's everyday culture. Take a famous fashion chain store: passing by one of their shops a foreigner could be forgiven for thinking it had gone out of business. All that can be seen is a large marble slab with a single black garment on it, the racks of clothes for sale are kept out of sight so that they may not intrude on the elegance of the bare interior.

Or take the following television commercial. A view of a wall full of windows fills the screen; as the camera pulls gradually back it is revealed to be the side of a tall American skyscraper; a single long strip of paper falls from the sky, twisting and turning as it drifts down through the centre of the screen — and is then replaced by the name of a brand of mayonnaise, without any apparent connection to the images that have gone before.

What this commercial illustrates is not just a partiality for minimalism, but a second trend also seen at work in the Expo, perhaps best caricatured as a "disinterest in facts". However many commercials you see on Japanese television, you would be hard put to find one that gives you any facts about the product — or even a good reason why you should buy it. As an advertising agent who boosted Sony's Walkman to success put it, "we stress the emotional benefits of the product, not the physical properties". Everything aims at the evocation of an ambience, a mood to be associated with the product — not at conveying information about the product. Indeed, Western society is often described as too oriented to facts, what the Japanese call "dry". Instead, they are (or like to think of themselves as) "wet", as being primarily concerned with their emotional reaction to things.

At individual Japanese pavilions in the Expo, the tendency towards extremes of understatement and over-statement can be clearly seen. So too can the tendency to rely on emotion rather than logic. For Westerners the chief criticism of the Expo would be that it dazzled rather than informed. One suspects that many Japanese would not really care. A Japanese corporate participant apparently shrugged off comment of this kind with "However educational and profound the theme may be, it's a waste of time and money if no visitors come". So the emphasis is on spectacle, fun for all the family, and slogans. There is another important consideration; the crowds are expected to be so huge that if anyone stops to press a button or think too long it might bring the whole Expo to a halt!

Nevertheless, there are many thought-provoking exhibits at the Expo and some of the best (for this reviewer) are described in the pages that follow. Pavilions of foreign countries, too, present their own individual styles — many of them the "high information" style that is more common in the West. It will be interesting to see how the Japanese react to them, and how the million or so foreign visitors expected to come react to Japanese exhibits. All in all, worth coming a long way to see. □



Japan Airlines' experimental High-Speed Surface Transport, on view at Expo, uses magnetic levitation for lift and a linear induction motor for propulsion at up to 300 kilometres per hour.

The theme: science cool and wondrous

THE spirit of refined taste is one facet of Japanese culture that is seen at its best in the government's Theme pavilion. There is no clutter, nothing obtrudes and there is often a remarkable sense of peace; even the couple of monitors showing videofilms do not sit clumsily by displays, they recline in elegant glass cases as though exhibition pieces in their own right.

The first hall, Sun and Water, is high, light and airy and contains what has become through intensive media exposure the star of the Expo: a giant tomato plant. Grown from just one seed, the plant will demonstrate the potential of hydroponic farming techniques; if all goes well it will reach ten metres in height and produce 12,000 tomatoes by the end of the Expo. So far it is growing on schedule, and managed 30 tomatoes for the press preview two weeks before opening day.

The second hall, Man, is more surreal. In semi-darkness on a central octagonal platform, a silver, metal man-like robot plays the organ (a little soullessly) from sheet music read by a single television eye set in the centre of its head. To one side, wall panels show an ever-changing pattern of nude human bodies — the female conveying "grace, beauty and warmth" and the male "strength and vitality"; to the other side a quadruped robot looking like a gigantic black ant crawls slowly up a staircase, a frill of sensors around its feet feeling for the next step; behind paces a strangely truncated bipedal robot — just a pair of walking legs ending in a squat metal box.

The last hall, Outer Space, is dark and cathedral; images are projected onto a screen from an Earth satellite while a golden model of it rotates silently alongside. On a moving walkway, one sails slowly across a vast elongated screen filled with images of molecular structures, planets, suns and nebulae that turn into the radial arms of colossal space stations. The journey ends in a corridor of sloping mirrors and bright red fine scaffolding that gives one the impression of having landed inside an infinite cubic lattice.

Along the way there are quite a few objects: the Shinkai 2000 deep-sea vessel hangs in the "Sun and Water" hall, and the silver multiple compound eyes of the solar ray concentration and transmission system stand at its centre. An NMR scanner, a laser scalpel and an organic holography display stand quietly in the "Man" hall. The second stage of the HI rocket has a corner in the "Space" hall. A separate open area — a future town plaza — in elegant greys has a model solar house and a city of the future.

But the real emphasis is not so much on things or on how they work, or on presenting a glimpse of the future; it is more to create an almost religious state of awe. Whatever science and technology is, it is something strange and wondrous. □

History pavilion

A little judicious rewriting

GIVEN the overall aim of the Expo to create a new feeling of warmth towards science and technology in the hearts of Japanese citizens, it is not surprising that the view of the past should require a little revision. In the process, some old and misleading myths about Japan have been thrown out.

What the first half of the government's "History" pavilion does is to chart factors responsible for the first Japanese miracle: the very rapid modernization of the country after it opened its doors to the West and abolished the Shogunate in 1868. Indeed, progress was so fast that within the space of a few decades Japan went from an isolated feudal country to a major power that was capable of defeating the might of the Imperial Russian army and navy.

Understanding of this leap forward is particularly important for developing countries, especially those in South-East Asia, who frequently try to take Japan as a model for their own development. The exhibition makes it clear that success did not spring from unprepared ground and was not due to some inexplicable spirit of the Japanese. First and foremost, even before direct contact with the West, Japan had a population that was highly educated and literate. Information panels (unfortunately written only in Japanese and thus inaccessible to foreign visitors) show how there was a major boom in small *terakoya* schools which gave basic education in reading, writing and the abacus in the early nineteenth century; by the middle of the same century it was almost certainly true (as it still is) that the Japanese were the most highly educated people on Earth.

It was to this relatively enlightened population that the best of Western technology was brought in to be imitated and improved upon. A series of intelligent decisions were then made to allow technology to disseminate quickly; some of the very first university engineering departments in the world were opened, systematic methods of measurement were introduced, a patenting system set up, and industrial standardization was adopted early on.

The defeat in the Second World War destroyed the material fruits of this effort — then came the second economic miracle and Japan's current technological successes. Explanations for these are much more abstract and fall back into clichéd explanations about the "special characteristics" of the Japanese people.

Beautifully arranged exhibits of traditional laquerware alongside the Sony Walkman show the Japanese sensitivity for "lightness"; a stylized Zen garden illustrates the Japanese desire to "control time and space"; a *karakuri* doll — a kind of sophisticated mechanical doll worked by levers which was first produced in the seventeenth century — is shown with a caption which links past interest in these dolls to the modern achievements in robotics (the "robot spirit"?). Japan is one of the few countries to endlessly dissect its "national spirit" to define its unique characteristics — the doll exhibit is as silly as one would be that put Victorian English miniatures alongside microchips (the technology was invented in the West, not Japan) to prove a British "love of the small".

Curious too is an exhibition of traditional technologies. A staggering set of six multiple projection screens with an overpowering sound system dominates a great dimly-lit hall at the back of which giant water wheels slowly turn. On the screen, flashing images of traditional sword-making, and from the loudspeakers a mighty cry as the red-hot sword is plunged into clear water. The message is a very fair one; that in the past Japan had independently developed sophisticated technologies. But the exhibit captions go beyond this and seem to suggest that there is a native Japanese science and technology, a scientific tradition that is uniquely its own; as though there were a difference between "science" and "Japanese science". Lying behind this there is perhaps some feeling that "science" is still in some sense an import and that proving there is a Japanese science will somehow make it more acceptable or lovable.

Having set out to destroy some myths about one miracle, it is a pity to see the exhibition introducing new myths along these lines. How much better the IBM (Japan) pavilion's attitude to history; Japanese scientists, as fine as any other nation's, take their equal place alongside foreigners in one international science. □

A touch of class from the British

LACONIC understatement, either a virtue or a character trait, marks the British pavilion. The designers have set out to ensure that visitors will not be impeded in their hunt for another stamp on their Expo passports by buttons crying out to be pressed. Instead, there is a cunning array of video screens hidden in structures as different as breaking waves and seashore rocks. Backward-looking (or historical) information has been avoided as if the plague. And to avoid carrying coals to Newcastle, the planners have made much less of the British government's domestic ambitions for information technology than might have been expected. But Sir Clive Sinclair has been persuaded (apparently reluctantly) to put the latest version of his Spectrum computer on display, while the UK programme for encouraging the use of computers in schools is heartily endorsed.

The first objective of the exhibit seems to be to let people know what Britain looks like, both to the eyes of visitors (accomplished by means of life-size cut-out models as in those of pop-up children's books) and by Landsat images collected from Earth orbit. If necessary, the exhibit will also demonstrate to visitors exactly where Britain is, apparently a matter on which many Japanese are uncertain.

Environmental protection, typified by the return of salmon to the Thames, looms large, as do some of the recent developments in medicine (NMR scanning, for example) and pharmaceutical research (H_2 antagonists for treating gastric ulcers, for example). The IRAS satellite is represented by a model that could well have been larger, but the infrared image of the Milky Way is as arresting as anybody could wish.

On balance, the impact of this modest display on visitors is likely to be pleasing and reassuring. Even before the video displays had been switched on, and the lights darkened, the sense of unpretentious orderliness was clearly apparent. Some may think that the decision not to look backwards notwithstanding, more might have been made of British contributions in basic research — in molecular biology, for example. But what, in any case, will stick in people's minds is the largest single object on show, a sleek Jaguar coupe in royal blue. Apparently the taste for these machines is catching on even in Japan, with the result that one is to be seen most days on the neighbouring parking-lot, the property of the manager of a nearby pavilion.

John Maddox

Pacific rivals

AUSTRALIA and Korea inhabit spacious pavilions, as befits fellow-Pacific states. But there the similarity ends. Australia plainly wants Japan to know where it came from. Korea's message seems to be that Japan should watch out for a rival to the West, and in the meantime spend its vacations in Korea.

Australia's history comes across in sepia-tinted blow-ups, with as full an account as anybody could ask for of the precedence accorded to the aboriginal population. Heroic eighteenth and nineteenth century settlers create a melting-pot to which Asian populations have since been added. Now there is a modest boast about Australia in astronomy, and about the keel that won the America's Cup from the United States. A dashing video display says perhaps only half of what might be said about the splendour of Australia as a place. Korea, by contrast, makes itself seem a little like Japan but more exotic (which is fair). Its high-tech products are relatively modest — cars, telecommunications equipment and so on. But the similarity is in itself enough to suggest the threat of competition from around the corner.

John Maddox

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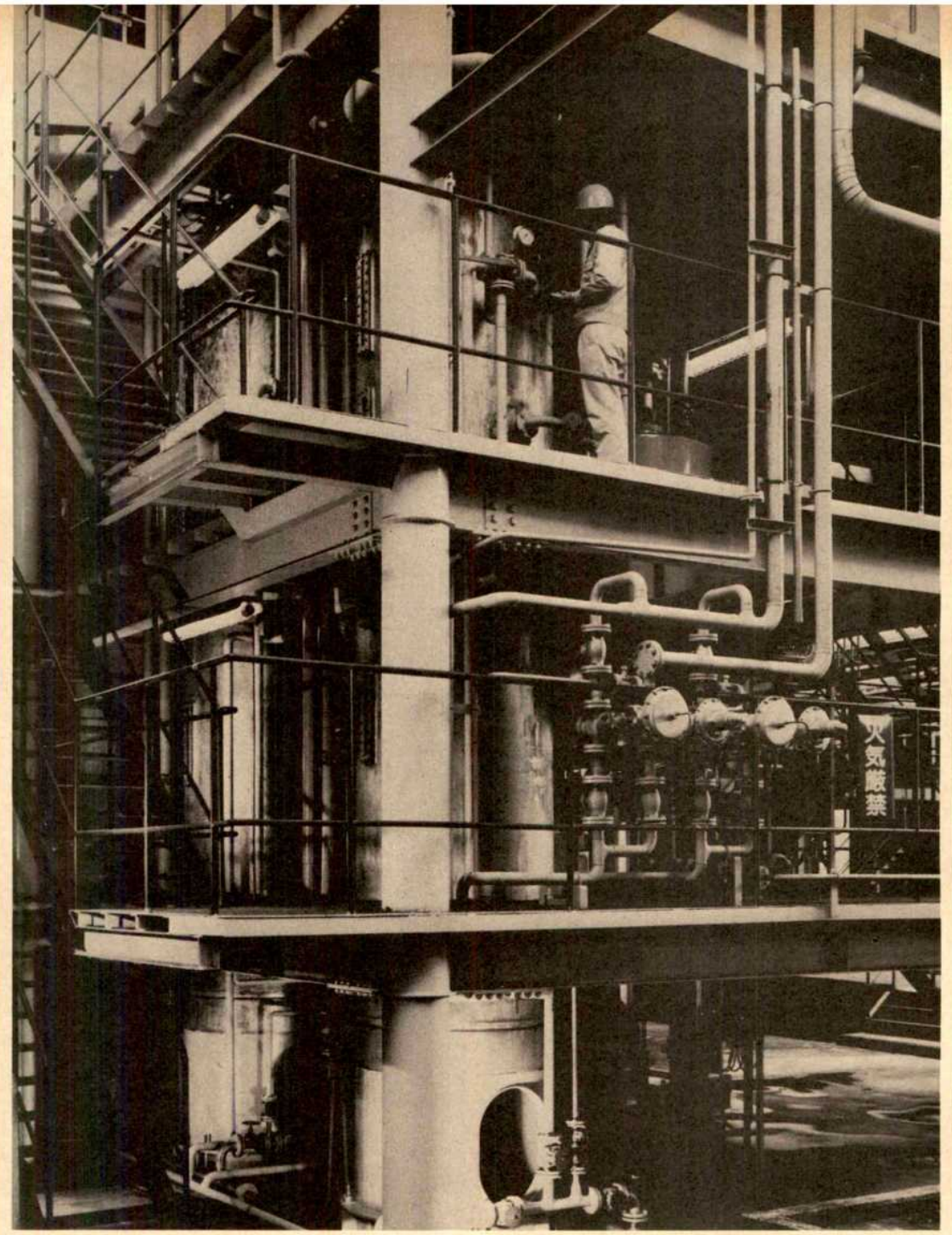
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Corporate pavilions

Electronic extravaganza

GOING over the top to prove that technology can be a barrel of fun are the big Japanese electronics corporations — Toshiba, Sumitomo, Matsushita, Hitachi, Mitsubishi, Mitsui and the Electric Power corporations. And for children and their mums and dads on a day trip to the Expo the whole experience probably will be great fun and a lot closer to a day at Disneyland than at a science museum.

The first three corporations offer films as their main attraction, the last three rides — a kind of high-tech ghost train — and Hitachi in the middle combined the two with a rotating movie theatre. Among the rides the "Electrogulliver" trip offered by the Electric Power corporations is by far the most unnerving.

The ride begins with thirty seconds of complete and total darkness, after which the chair makes a sudden lurching descent and its occupant is hit by a fine spray of cold water. This turns out to be preparation for a quick steam in a very hot volcanic magma chamber. After that the traveller goes through a forest inhabited by twitching purple cockroaches and into the heart of the Sun to participate in a nuclear fusion reaction — an environment available in 3-D and computer simulation in pavilions elsewhere, so the visitor to Tsukuba soon becomes perfectly at ease with a few protons flying excitedly about.

Elsewhere there are rides into space and past black holes, and in Mitsui's water theatre the adventures of a boy and robot, incorporating a film projected onto a giant circular waterfall surrounding the ride.

Indeed, exhibitors opting for film are nowhere content with mere conventional projection systems. 3-D is everywhere. Sumitomo boasts Japan's first purpose-built giant 3-D screen and a moving tale of a little girl and her sheepdog "Bozo", and Hitachi the world's first 3-D colour computer graphics and a curiously old-fashioned view of the future, with robots vacuum-cleaning and scolding children for not doing their homework in a story much like that of comic strips of 20 years ago. Toshiba has a super-reality theatre using film taken and projected at three times the normal speed for the ultimate image. Special effects are provided by the maker of those for "2001" and "Close Encounters of the Third Kind" and contain the best robot ("Pal") in the Expo.

Some pavilions also have some technology on display. Toshiba's robots are particularly impressive. One inspection robot and five one-armed assembly robots deftly spin traditional Japanese tops and make them walk a tightrope. Alongside is state-of-the-art medical imaging equipment — magnetic resonance, tomographical X-ray and ultra-sound — and an astonishing pattern recognition machine that can sort out foreign banknotes at the rate of 1,500 a minute. To make the machine more lovable for the Expo, it does not actually touch any money but sorts out five kinds of cards depicting scenes from fairy tales. Unfortunately the machine still looks for all the world like a giant metal-and-glass sarcophagus — a few pink ribbons might have improved it.

Most pavilions rely simply on spectacle. You certainly will not learn any science from them (or see much real technology) and Westerners are in danger of emerging with a fuse blown somewhere. But Japanese children have been reared on their local television and are made of sterner stuff. They will be queuing up for more until the Expo ends. □



Film projection onto falling water is Mitsui's main attraction.



Four million pairs of 3-D glasses will be given away during the Expo.

NEC pavilion

A place for galactic heroes

FUN and games with the future, the insistent theme of the Japanese pavilions, will be eagerly sampled at the Nippon Electric Company (NEC) pavilion, most easily recognized by the 32-metre parabolic antenna which towers above it. There was some embarrassment, on the day it snowed heavily at Tsukuba the week before opening day, that workmen had to keep the crowds away from the lumps of slush falling from the rim until the dish could be pointed to the zenith.

NEC has built a theatre for playing interactive computer games. Visitors are given a piece of plastic, not called a credit card but called a boarding pass, that will activate a small computer terminal in front of every seat. A film whose script begins with Neil Armstrong's observation on landing on the Moon puts the audience into a space ship whose navigators repeatedly run into swarms of meteorites and, now and again, a black hole. At the approach of each potential catastrophe, everybody's seat vibrates. Those who make the wrong choices of a strategy for escaping find that their seats tilt back alarmingly. Half-way through the show, there is an exercise at destroying meteorites with a ray-gun, when each terminal records its user's score.

Crude though it may be, this jazzy show cannot but be a hit. NEC has probably underestimated the queues there will be, with merely 30 square metres set aside outside for standing in line. But those who will be waiting under cover will be treated to a 5-minute quarrel between three television sets whose screens are not merely filled with cleverly drawn animated faces, but which can nod and shake so tellingly that even those who cannot understand the Japanese will think them alive.

NEC's motto for the present is "C & C", which means computers and communications. The company has set out to simulate something of what it thinks the next century will be like in what it calls its Techno-Plaza. There is a vehicle with a computerized guidance system that locates it on a moving map display, and which tells the driver when and which way to turn at intersections. Just as well, visitors will conclude, for the driver will otherwise be too busy using the tricks with which the vehicle is equipped for sending documents back to head office and retrieving data from the databanks. NEC apparently has no immediate plans to put its car on the market.

Two of the exhibits are more telling: a group of three six-jointed robots, or simulations of human arms, which are wired and programmed in concert to carry out co-operative tasks, and the prototype of a telephone interpreter that will convert spoken Japanese into spoken English. NEC has for some time been putting a large part of its research and development into artificial intelligence. If the rough edges of its interpreting telephone can be smoothed out, it may not have to wait until 2000 to have something to sell.

John Maddox

Fujitsu pavilion

Toughest robot in town

What mankind can dream, technology can achieve ... Robots act for us, machine translation systems think for us ... computer imaging technology simulates realities beyond our realm of perception.

ALTHOUGH Fujitsu falls in with most other corporations in its glorification of technology, it does so with a little more style; some interesting exhibits are on display and its 3-D film is streets ahead of anyone else's.

Dominating the exhibition is the world's largest humanoid robot, Fanuc Man; 5 metres tall, weighing 25 tons, and kept in a special cage which makes it look as though it is eager to break out and start climbing skyscrapers. But Fanuc Man is,



symbolically, both gentle and strong. For the amusement of visitors it alternates between the dexterous task of assembling a one-thirtieth scale model of itself, aided by television cameras set into its hands, and pressing a 200-kg barbell (not by sheer brute force but by careful load balancing). Fanuc Man and his like are destined to assist one-armed robots who spray paint and spot-weld cars with the assembly of heavy components — doors, seats and so on.

Japan's lead in robot production is pretty much unquestioned. But software has been considered a different story, with Japanese thought lagging behind their US and European counterparts in creative ability. Fujitsu makes that point but claims that the software used at the Expo shows just how quickly things are changing. Fujitsu believes its machine translation system to be the world's most advanced and it is set to work at the Expo translating Japanese sentences — written by children at a booth on their way into the pavilion — into English, French and German for projection on a screen elswhere in the building. A Fujitsu engineer was happy to admit, though, that even a short sentence takes 15 minutes of mainframe computer time to translate. Just why is not hard to see. Japanese is written without breaks between words and the first step must be to segment the sentence into words through a dictionary and information about the adjacency relationships of words; only then can a context-free grammar be put into use to parse the sentence and identify grammatical elements. Next comes the really difficult bit: the computer has to have some sort of commonsense world model to figure out which particular meaning of each word is intended — it has to "understand" the sentence — before a translation can be attempted. Fujitsu uses the semantic network approach; essentially a hierarchical grouping of words with linked meanings (a bit like the organization of a thesaurus rather than a dictionary), as a part of a knowledge base. It is after this stage that words from a different language with corresponding meaning can be selected.

Clearly the problems are very complex and general purpose machine translation with enough knowledge to handle the rambling sentences written by real people is a long way off. But in restricted fields where ambiguity has been deliberately eliminated, progress is expected to be more rapid. Within five years, Fujitsu expects computers to handle almost all translation of technical manuals; technical translators may be the first highly skilled white-collar workers to be made redundant by computers.

The other software advance of which Fujitsu is keen to boast is that used in generating the company's new 3-D film — the world's first 3-D computer graphics film for projection on a dome. The film depicts a journey inside ice crystals, through the planets, and into the Sun to see the mechanism of nuclear fusion, and ends with a sensational simulation of a DNA molecule being wound and rewound and re-



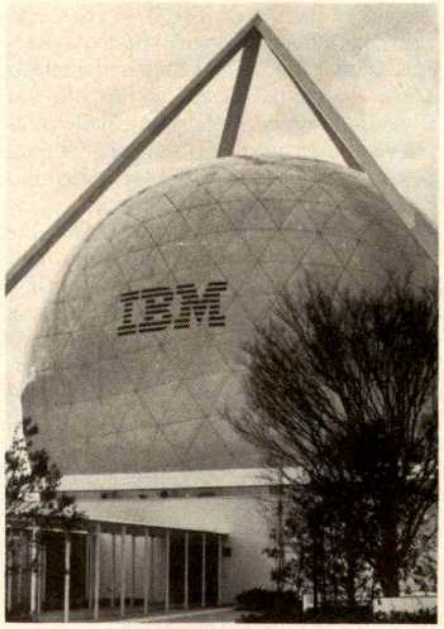
packed until it forms a chromosome. Days of computing time on one of the world's largest and fastest supercomputers were needed to generate this sequence; the intention was not to produce a cartoon but to produce exact simulations based on the most recent molecular models. Many a 3-D film at the Expo promises a trip inside a "different dimension" — Fujitsu's is the only one to achieve it. □

IBM (Japan) pavilion

A touch of wisdom

AMONG the domestic pavilions, IBM (Japan) would have to take the prizes for exhibits that are both fun and teach one something about science. Responsible for the overall organization was Nobel-prize winner Leona Esaki, who now works for IBM, and a team of top Japanese scientists—the difference shows. What IBM wanted to get over was something of the spirit of scientific discovery and of the way each scientist builds on what has gone before. The latter is attempted through a picture and text wall display of key periods and individuals in the history of science. The earlier periods — the Ancient Greeks beginning with Pythagoras, passing through the contribution of men like Avicenna from the Arab world to the Middle Ages and the revolution brought about by Brahe, Kepler, Galileo and Newton — were pretty much what might be expected. From then on a newer view of the history of science emerges.

First, there is the transition in the early eighteenth century when "the religious motivation to demonstrate God's will through the understanding of Nature's laws gradually gave way to the study of Nature for its own sake, or... for material benefits" — the age of Watt, Franklin and Cavendish. Then follows the Age of Specialization, that of Darwin, Maxwell and Edison, when scientists founded professional societies and "sought recognition through short papers on specific topics rather than lengthy treatises attempting to explain all natural phenomena as elements



The IBM pavilion takes its form from paintings of the nineteenth-century Zen priest Sengai — the circle, triangle and square respectively symbolize the Universe, human intellect and Earth.

Instruments of a New Age of Exploration

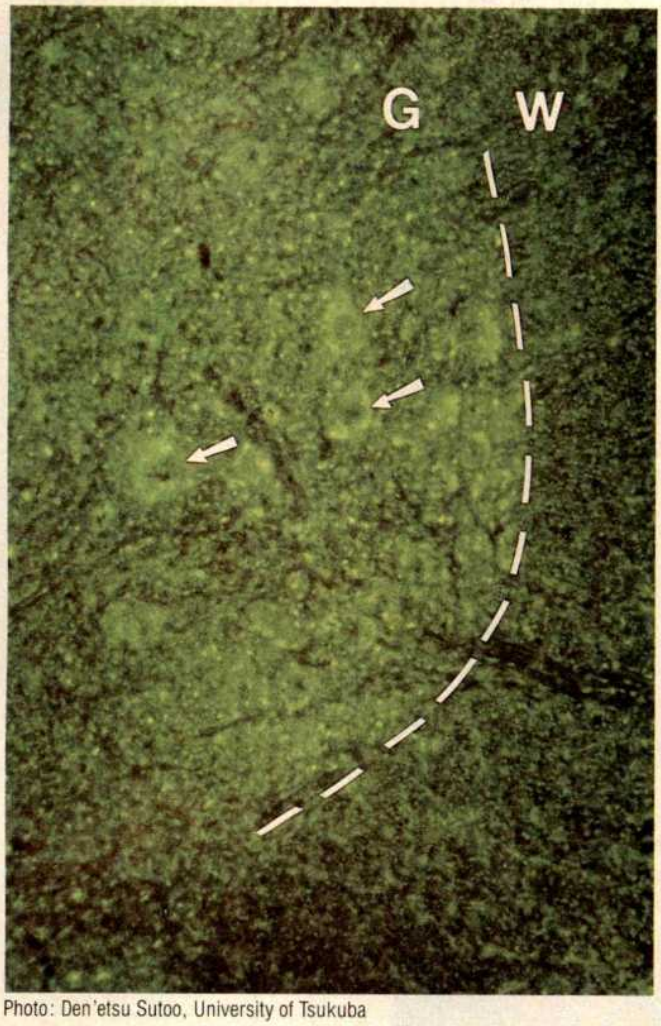
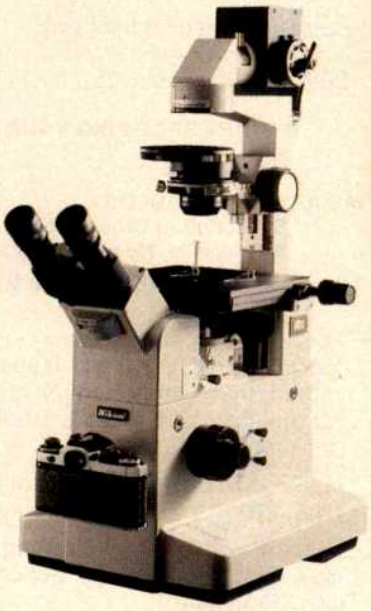


Photo: Den'etsu Sutoo, University of Tsukuba

The ongoing challenge of nerve research—potentially one of the most rewarding research endeavors in advanced biochemistry. Nikon brings you this brief glimpse of how man and machines are working together to deliver new views of the nerve world.

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of a single system". This was the period when *Nature* (the journal) was born — not that all its papers were that short.

In our own century came the Age of Technology, when immediate industrial applications came to be expected for research results and "military research in particular flourished" — a remark that would be commonplace if it were not the only reference to the military uses of science spotted in the whole Expo. And now we are in the Age of Big Science.

Along the way, Japanese scientists who had made a contribution to the world's science are introduced quite naturally, in contrast to the government's History Pavilion, which attempts to establish that there is a "Japanese science". The four Japanese Nobel-prize winners, Yukawa, Tomonaga, Fukui and Esaki himself, are of course mentioned but so are others who are not well known in the West but should be: among them Shizuki Tadao (1760-1806) who introduced newtonian mechanics to Japan, Kitasato Shibasaburo (1853-1931) who discovered the plague bacillus, and Nagaoka Hantaro (1865-1930) who created a Saturn-like model of the atom.

To teach you something of what science is about there is a whole series of interactive displays of the type familiar in Western museums, with plenty of buttons to press. In a model of the atom, for example, it is possible to excite an electron into higher orbitals and see it emit a flash of light as it later drops back; on a giant globe one can manoeuvre continents around to follow different stages of the Earth's evolution. Scientific games are also available in plenty — played on a whole roomful of IBM personal computers.

The supposed highlight of the exhibition is a theatre in which 11 synchronized projectors show a film simultaneously on a domed roof and a giant sphere set in the middle of the hall, while spectators are carried around in a circular walkway. The film is designed to teach the relationships and dependencies that exist between man and science and not "simply to dazzle the visitor". All very proper and almost a rebuke to some pavilions nearby. □

NTT pavilion

Electronic future on display

JAPAN'S soon-to-be-privatized telecommunications monopoly, NTT, has seized the opportunity provided by the Expo to try and show that "INS will make life more exciting".

INS (Information Network System) is what NTT believes will be the telecommunications system of the future: optic fibre cable and complete digitization of the transmission systems will usher in the electronic age of videophones, home shopping and banking, instant access to databanks and electronic mail. A model system is already on trial in the Mitaka suburb of Tokyo (see *Nature* **311**, 289; 1984); the very newest gadgets were on display at the Expo.

One instrument not seen before is the scopephone; a telephone with a couple of centimetre-square black-and-white video screens in its plinth and a miniature camera, revealed only by a small opening, under that. The scopephone does not need the high-bandwidth cable that handicaps ordinary videophones or videoconferencing facilities; of course, the picture is not of such high quality, and if the subject moves about too much the picture temporarily blurs before settling down again. But the scopephone gives a perfectly adequate sense of seeing the person you are talking to — and it is cheap. Chances are this could be commercialized on a large scale very soon.

Also new is a miniature fax (facsimile transmitter) built into a public telephone that achieves the transmission of whatever is written on a small piece of paper just the size of a visiting card; it will make it possible for the Japanese to extend their habit of introducing themselves by exchanging namecards even to a telephone call. As most Japanese bow even when speaking on the telephone (the habit is easy to acquire and hard to break) this invention will make the ritual introduction complete — clearly someone in NTT's marketing division had a brainwave.

On display too is a videoconference room, the sketchphone (that enables one to transmit doodles as one speaks) and the CAPTAIN teledata system. But the big problem with all the hardware is a familiar one — what can it be used for? NTT tackled this question by asking what would have happened if the hardware had been available a hundred years ago: a series of sketches performed by actors in small studios around the pavilion gives the result.

One performance comes right out of one of the most curious episodes in Japanese history soon after the Meiji restoration had opened Japan to the West. The setting is the Rokumeikan — a banqueting hall and ballroom specially built in 1883 for the Japanese elite to entertain foreigners to Western-style balls in formal Western clothes. The balls must have been very uncomfortable for the Japanese but their purpose was very serious — to convince the long-nosed red-faced barbarians who had pushed their way into the country that the Japanese were as civilized as anyone else. At issue were the "unequal treaties"; treaties forced on the Japanese that ensured that foreigners who committed crimes in Japan could only be tried (or more often acquitted) by their own consular courts. The treaties were a source of great shame to the Japanese. In the event, a naive display of elegant manners and correct dress did not convince the foreigners that the Japanese were equals and the treaties were not revoked — rapid industrialization and military might did the trick a little later.

So much is known to every Japanese schoolchild; what the sketch shows is poor Mrs Itoh, wife of the first Prime Minister Itoh Hirobumi, worrying over what to wear, and how to dance, and what food to cook to please the foreign devils at the Rokumeikan. Of course, INS provides all the answers — instant access to video databanks through the high-speed video response system gives her dancing lessons and cooking lessons and doubtless many other insights into all our nasty habits. Information is power! The point is well made: Tokyo is these days full of foreigners clutching books on how to do business with the Japanese (and even how to hold chopsticks) and doubtless wishing there were video databanks to make it all a little less painful.

But NTT's video presentations of the future uses of INS fall short of being really convincing. A forester worried about how many trees to cut calls up an Earth resource satellite, a fisherman with access to a meteorological satellite — will it be cost-effective? With INS's more exotic applications, Japan has for once moved out in front of other nations. Will Japan discover that being a trail-blazer can also mean going the wrong way? □

Brainwaves to touch

WHY should Kodansha, a major Japanese publisher, have taken an area of 1,800 square metres at Tsukuba to build what it calls a "brain house", a multi-video explanation of how the human brain works? Because that is how people use its products, of course.

The result is an extraordinarily ambitious exercise in popularization. The designers have tried to represent what little is known of the anatomy and neurophysiology of the human brain in a series of working models; press a button and see how input to the right eye distributes signals predominantly to the left cortex, but partly to the right hemisphere. Other sensory systems, and the role of the hypothalamus and the pituitary, are similarly described. Perhaps the definiteness of the labelling (and the life-size and thus too-small models) may give visitors the impression that phrenology is back in fashion.

This sober stuff is submerged in one of the jazziest video displays at Tsukuba. The whole of a darkened dome is used to represent the way in which the networks of neurones are used in different physiological functions, while a character called Goku, derived from Chinese fairy stories but animated for these purposes, serves as an entertaining unacademic expositor.

As popularization, the pavilion succeeds in the sense of showing a lot of what is known. It conveys a sense of what remains unknown less convincingly. But it is a courageous attempt to explain a large chunk of science without promising that a host of gadgets will ensue. John Maddox

TDK pavilion

Animals getting taped

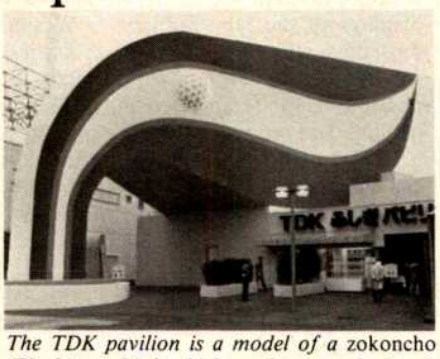
MAKING strange the ordinary abilities (or events) we normally take for granted is perhaps the first step to becoming a good scientist: one Nobel-prize winning ethologist used to advise that one should try looking around one from the standpoint of a "Martian that just arrived on Earth". The pavilion presented by TDK (the magnetic tape manufacturer) comes close to simulating that spirit. It attempts through a film to give some taste of the strange experience of perceiving the world as if through the visual, auditory and touch senses of an animal.

The idea is one that has often attracted animal documentary film makers but is notoriously difficult to carry off. TDK's attempt is at its most successful in depicting the underwater world of fish, where changes in the field of vision, depth of focus and the absence of long range are easy to simulate photographically. The world of the horse, where the visual field is extended to 320 degrees in the horizontal plane but greatly narrowed in the vertical plane, is also easy enough.

Things get much more difficult when smaller forms of life are considered, the forms of which D'Arcy Thompson wrote: "A water-beetle finds the surface of a pool a matter of life and death, a perilous entanglement or an indispensable support. In a third world, where the bacillus lives, gravitation is forgotten. We have come to the edge of a world in which we have no experience and all our preconceptions must be recast."

Alas, after attempts to see what a rainstorm would be like for a small insect — an occasional encounter with a very large object — things come seriously unstuck as the film turns to the compound eye; an extraordinary kaleidoscope of multiple images fills the screen while a voice tells one that "it is thought the brain may process the images into one" (of course, this may have been a clever in-joke for troubled insect neurophysiologists). Nor does the film find any new way round the intractable problem of modelling auditory and tactile senses, other than explaining how they are better developed in certain species.

But overall, the film just about works, and at the end it returns nicely to technology by stressing how, from the study of animals with senses superior to our own, we can learn how to build new sensors. Respect Nature and learn from it is the message, one that got lost in the multi-sensory stimulation of most pavilions. □



The TDK pavilion is a model of a zokoncho *(Elephinsect bird) which combines an elephant's trunk, an insect's compound eye and the flying abilities of a bird.*



Tokyo residents kept awake by the next-door neighbours' stereos would certainly be grateful if Matsushita can speed development of their speakers. Sound from the large circular speakers set a couple of metres above the ground can only be heard when standing within the smaller circle seen on the floor — a 50-centimetre movement of the head and all the sound vanishes. Normal sound is of too long a wavelength to be sharply focused in the same way that light can. Only ultrasound can be easily focused — but of course humans cannot hear it. So what Matsushita have done is to use two focused beams of ultrasound of slightly different frequencies. At the point of focus they interact with one another; the result of the sum and difference of the two waves is the generation of audible sound at that point only.

Suntory pavilion

Today birds, tomorrow men

AT last a discordant note in the hymns being sung to the boundless joys of technology at the Japanese corporate pavilions! At the Suntory pavilion not only is it acknowledged that technology can cause plenty of problems, but also that some of those problems need dealing with fast.

Suntory's must be the only pavilion to state clearly that curses have also sprung from technology: "ever worsening environmental pollution, wanton consumption of the precious blessings of Nature, a loss of concern for the protection of living things, a lack of warm regard for one's neighbours".

The pavilion's theme is "Today birds, tomorrow men", the point being made is that birds are among the living things most sensitive to changes in the environment and many have been pushed to extinction — after them it is us. To drive the point home, the pavilion employs the world's largest cinema screen and a film, concentrating on efforts to save wild birds, which contains what must be some of the best shots ever taken of wild birds in flight.

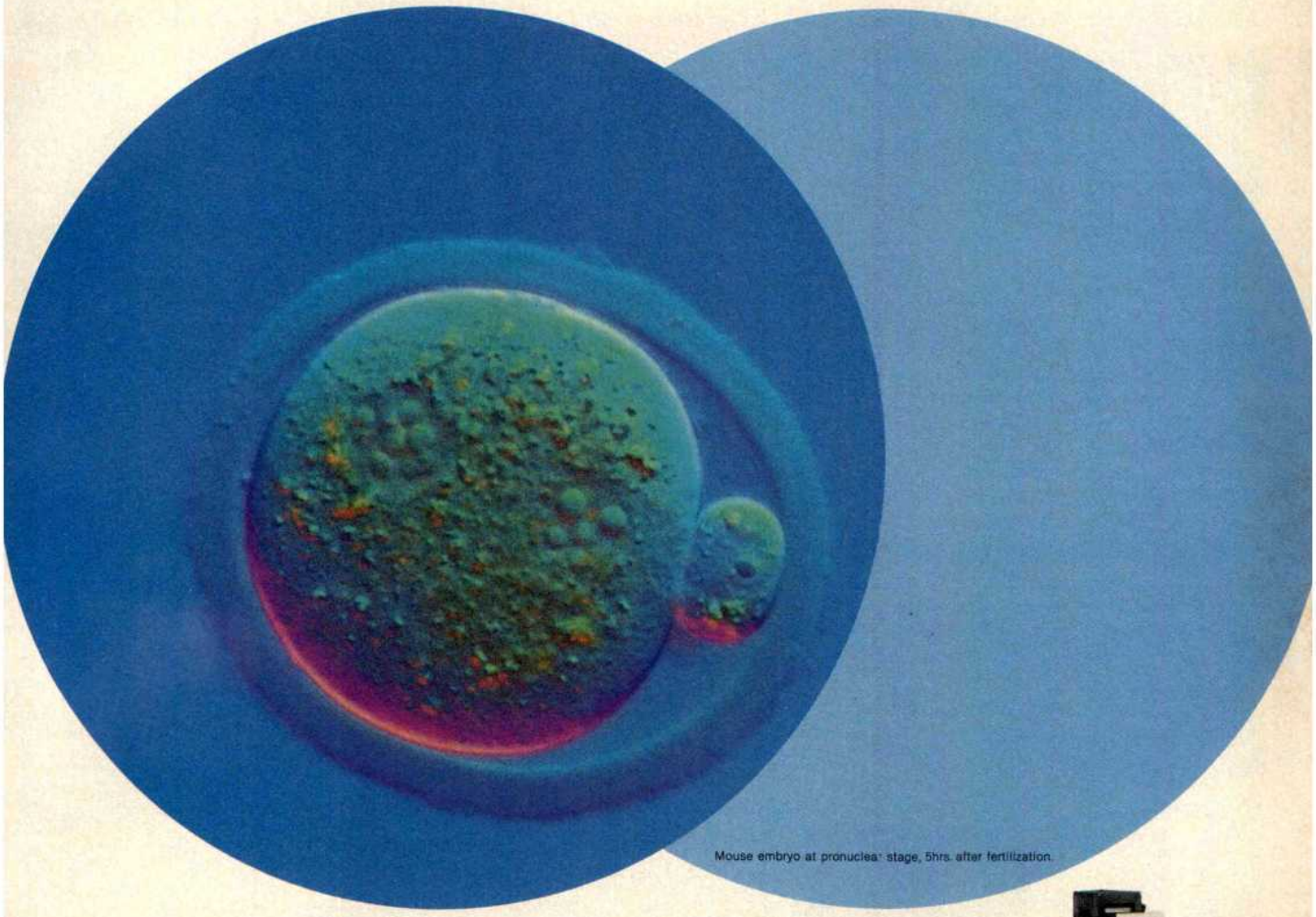
The film centres on a family of Canada geese and, in the most striking sequences, it follows them in flight across ocean and forest, partly in extreme close-up from just behind the leading bird as the flock flies in a V-shaped formation soaring across the oceans. How did the photographer manage it? A Suntory representative could say only that a special "silent glider" had been developed by a Japanese-Canadian team to allow the mid-air close-up.

To its credit, the pavilion is part of Suntory's Wild Birds Campaign, which has been running since 1973. Suntory is a large whisky manufacturer and has built bird sanctuaries at many of its plants. No doubt it is good for the corporate image, but it is good for the birds too. □



Suntory's pavilion was shaped by bending steel pipes, one by one, into catenary curves. The surfaces are meant to evoke the image of a hill and to symbolize Man's desire to regain the beauty of the natural environment of the past.

A look into the future



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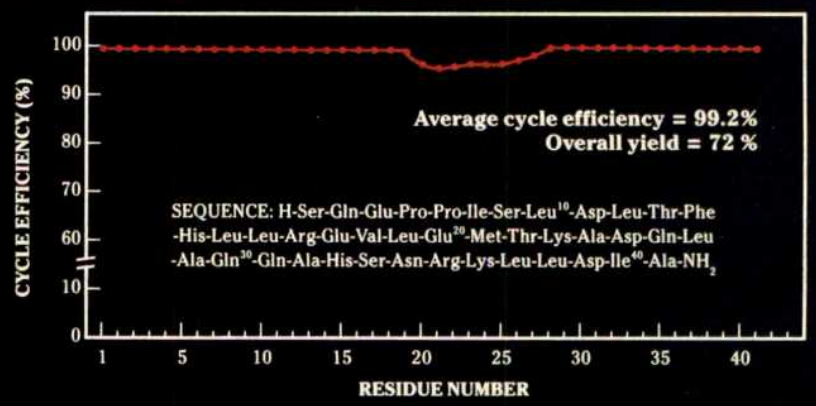
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CERN collider seeks the Centaurs

Last Sunday the proton-antiproton collider at CERN generated the world's highest energy interactions yet, in a quest for some strange effects first seen in cosmic rays. The work should continue.

AT 1 a.m. last Sunday morning, jubilant experimenters at the European Organization for Nuclear Research (CERN) working on the UA5 detector on the proton–antiproton collider recorded their first events at 900 GeV in the centre-of-mass, the highest energy at which matter has been collided with matter (or antimatter) under controlled conditions on Earth. The object over the next few weeks will be to discover if the collisions show signs of the very-high-energy "Centauro" ("centaur-like") events first detected in a Japanese–Brazilian cosmic-ray experiment in the Andes[1] as long ago as 1972, events that appear to lack all production of photons. Centauros are unexplained and treated with scepticism by most experimental particle physicists, but if they exist — and those who have studied the data carefully say they do — they would presage a fundamental reconstruction of theory.

Hence CERN's brief gamble, which, following an idea by UA5 group leader John Rushbrooke of the Cavendish Laboratory, Cambridge, involves a complicated ramping of the bending magnet current and accelerator cavities so that the colliding beams can reach — momentarily — 450 GeV apiece. The energy can only be maintained for four seconds at a time, as the magnets would overheat at the currents required to sustain the bending of 450-GeV beams. So the beams are decelerated to 100 GeV, and then brought up again to 450 GeV a few seconds later when the magnets have cooled sufficiently. It is undoubtedly an extraordinary feat of accelerator engineering, which pushes the collider energy up nearly by half from its usual constant 630 GeV maximum in the centre of mass.

The experiment certainly is a gamble, however, and CERN is treating it as such, giving UA5 the tail-end of the electricity supply year, when by contract *Electricité de France* can cut supplies to CERN for a day at a time — and usually does. There are five such *jours critiques* left before Easter, when UA5 must shut down for good. CERN staff describe experiments at such times as "crazy runs", but Rushbrooke said on Monday that he hopes UA5 will still collect some 100,000 events.

This should be enough to see if the Centauro effect is real — provided it occurs at or below 900 GeV. UA5 has already searched at 630 GeV but found no sign of the events. In the cosmic-ray experiment on Mt Chatacaya in the Andes, however, a per cent or so of the highest energy events recorded were photon-less Centauro events. (These events appeared unusually different in the upper and lower halves of the Chatalcaya detector — hence the "centaur" designation.)

Nevertheless, all the five Chatalcaya Centauros appeared to have equivalent centre-of-mass collision energies of 1,500–2,000 GeV, and it is uncertain whether, even at its boosted 900-GeV energy, the CERN collider will reach the threshold at which the Centauro effect sets in.(It is presumably somewhere between 630 and 1,500 GeV, if the events exist at all.)

At this point CERN's competitor steps into the act — the Tevatron collider at the Fermi National Accelerator Laboratory (Fermilab) near Chicago. Already this July and August, the superconducting Tevatron will take its first trial proton and antiproton beams, and the detector designed by Roy Schwitters of Harvard University and his collaborators will begin to take data at centre-of-mass energies that should run from 800 to 1,600 GeV. "It's a shakedown cruise" and "a very ambitious goal", said Schwitters last week, to get data on the Tevatron collider so early (the first serious data were not expected before spring 1986) and "we'll be delirious with joy if we see any collisions", but the chance is there that if CERN sees no Centauros now, Fermilab may see them this summer at its higher energies, or at least by the end of next year.

UA5 is worth a gamble, however — and not only to keep up the prestige of CERN (though prestige itself is not a negligble factor, as the vitality of a laboratory depends on it). UA5 is a unique detector, containing the world's largest "streamer chamber". This visualizes tracks as streams of sparks, rather as bubble chambers visualize them as rows of bubbles, and can resolve more detail in multitrack events than many other detectors, such as those under construction at Fermilab.

If, as the cosmic-ray experiments suggest, some strange global change is going on in the track composition and multiplicities at very high energies, UA5 may be in the best position to detect it. CERN might even be well advised, if the present run reveals no Centauros, to try boosting the energy yet further in the next "crazy run" period in March 1986, by improving the cooling of the bending magnets (though that could be expensive). Fermilab, also, might do well to introduce steamer chambers to its complement of Tevatron collider equipment.

But this leaves out the question of the cosmic-ray observations themselves, and whether more can be done in that area. Undoubtedly physics smacks of the 1940s rather than the 1980s, as the events are rare and uncontrolled — as opposed to the billions of events that can be produced with tuned, intense accelerator beams. But they can be of almost unlimited energy, and still thus throw up new physics, as the Centauro events themselves indicate.

One member of the UA5 collaboration, Narendra Yamdagni of the University of Stockholm, has spent five years working with the Japanese physicists who contributed to the Centauro study, and is impressed with the evidence. This shows up not only Centauros, but also other ultra-high-energy phenomena such as "binocular" events, where the cosmic-ray collision far up in the atmosphere appears to produce two (or more) clear jets of products, and, more recently, so-called "chiron" events in which there appears to be a microscopic bunching of tracks within each jet. A Moscow collaboration working on Mt Pamir has also seen a Centauro event "even more spectacular" than the ones seen on Chatalcaya, says Yamdagni.

Yamdagni has now left cosmic-ray work to experiment on UA5 and the large electron collider (LEP) at CERN, but he strongly recommends that cosmic-ray research should be amplified, perhaps by flying 1-ton cosmic-ray experiments in routine passenger-carrying jumbo jets. "Their payload is 50–70 tons" says Yamdagni, "so it should be possible to accommodate a ton".

Cosmic-ray intensities in the stratosphere are 40 times those obtainable deeper in the atmosphere on Mt Chatalcaya, Yamdagni points out. Moreover, within a one-ton experiment, equipment (such as proportional chambers) could be included to make results less ambiguous than at present. It would also make them more acceptable to sceptical particle physicists. Japan Airlines are already flying, free, 20-cm-cube detectors designed by the Waseda University group that helped find the Centauros. Perhaps they should be 125-fold more generous, and extend the dimensions to a metre. **Robert Walgate**

1. Lattes, G.M.C., Fujimoto, Y. & Hasegawa, S. *Phys. Rep.* **65**, 151 (1980).

Publications

Variations on a theme

from Jared Diamond

WE SCIENTISTS have chosen a cruel profession. To advance, we must publish, but our best works are often rejected, published in mangled form, ignored, or criticized. We must publish a lot, but not by plagiarizing the work of others nor by recycling our own material. While chasing grants, claiming priority for discoveries, and currying academy memberships, we risk missing deadlines, being passed over for appointments, or getting fired. We suffer grievously from politics, and few of us get rich. Worst of all, only a short span of productivity remains to us from the time we complete professional training until our creative powers wane. How are our problems to be solved? On the occasion of the 300th anniversary of Bach's birth (21 March 1685), what perspective can be gained from the way great composers faced these problems?

Incomprehensibly to modern scientists, desperate to amass long lists of publications, Bach never bothered to publish his B minor mass, St Matthew and St John passions, and Brandenburg concerti, which rank today among the monuments of western culture. Those works survived in manuscripts; his great C minor fantasia and fugue survived in a single copy, supposedly rescued from a pile of wrapping paper in a Leipzig shop; but his St Mark passion and dozens of cantatas mentioned by his contemporaries disappeared.

Other composers, eager to get their works published and decently produced, have encountered difficulties that are familiar to scientists. A few weeks before Schubert's death, four of his greatest works — the C major quintet and the last three piano sonatas — were rejected in one letter from the Leipzig publisher H.A. Probst, Beethoven's violin concerto was premiered without a rehearsal, and the soloist (one Franz Clement) interrupted it to play his own sonata, on one string with the violin turned upside down. Verdi could not complete *Aida* in time for the deadline (to celebrate the opening of the Suez Canal).

As for plagiarism, Mozart's requiem was commissioned by Count von Walsegg zu Stuppach, who liked to order works from professional composers and produce them as his own. The art of recycling works of one's own and others was honed to perfection by Bach and Handel. Of Bach's fourteen concerti for harpsichord, not more than two are original, while the Christmas and Easter oratorios were mostly lifted from his cantatas. Scientists have lost their jobs for far less extensive recycling.

Virtually all great composers have been exposed to vicious criticism. Berlioz despised Bach's music; Debussy, Fauré and Dukas walked out on Mahler's conducting of his second symphony; Weber viewed Beethoven's seventh symphony as evidence of insanity; Brahms compared Bruckner's symphonies to endless serpents; and a critic likened Pasatieri's music to a "stream of perfumed urine". Compared with such metaphors[1], the invective of the most pugnacious modern biologists — the cladistic taxonomists and vicariance biogeographers — is unimaginative (for example, "Darwin preached from ignorance"[2]). Like the research of Mendel and Wegener, Ives's work found widespread approval only after his death.

Disappointments in the quest for positions and honours have loomed as large for musicians as for scientists. Through servile letters, Bach managed to wheedle the honorary title of royal court composer out



"... I am afraid we shall only be able to publish your manuscript if it is cut by ten bars..."? (British Library).

of the Elector of Saxony. Other composers suffered crushing rejections. Few scientists take the loss of an NIH grant as hard as Tchaikovsky took the termination of his 14-year grant from the widow Nadezhda von Meck; he wrote bitterly that her name would be found carved in his heart when he died. To the end of his life, Brahms remained deeply wounded by the action of the Philharmonic Orchestra in Hamburg, his birthplace, in passing him over at the age of 30 as conductor. Schumann was compelled to resign as conductor at Düsseldorf, while the Vienna Opera fired Mahler in 1907 and Richard Strauss in 1924. A worse fate nearly befell Haydn, who as a teenager narrowly escaped castration by a choir director eager to preserve his soprano voice.

One might suppose a musician's career to be free of the problems of priority that are commonplace to scientists; a race to isolate the AIDS virus, yes, but surely not to compose the B minor mass? In fact, the shortage of suitable libretti has provoked scrambles among opera composers rivalling that which pitted Pauling against Watson and Crick. Leoncavallo, who apparently wrote an operatic libretto based on a novel by Mürger and showed it to Puccini in 1893, was enraged to learn from Puccini over lunch in 1894 that he was busy at work on a rival libretto. Newspapers reported both composers' intentions that night, and the race was on. Puccini won by 15 months; he also wrote the better version of *La Bohème*.

Like great scientists, few great musicians become rich, and fewer still are shrewd enough to retain their riches. Bach's Brandenburg concerti were sold after his death for four gröschen apiece; Beethoven lost money on the premiere of his ninth symphony. Brahms eventually was able to sell his compositions for high fees but foolishly entrusted management of his money to his publisher Simrock, who lost heavily in stock market speculation.

Musicians have had even less success than scientists at using their talents for or against political causes. Prokofiev dutifully composed a cantata, *Hail to Stalin*, and was named People's Artist of the Soviet Union, but lived to see his works banned, like those of Lysenko's rival, the geneticist Vavilov. Shostakovich fell from official grace and regained it with his fifth symphony (subtitled "A Soviet Artist's Reply to Justified Criticism"), only to fall again.

Comparisons between scientists and musicians are especially instructive for psychologists studying how human creativity develops with age[3-7]. Creativity tends to flower and fade early in fields that require reasoning skills rather than varied knowledge, but late in fields requiring one to unite disparate data. Thus, pure mathematicians and molecular biologists peak in their twenties and thirties but evolutionary biologists peak much later. Darwin published *On the Origin of Species* when he was 50, and produced seven major books in the eleven years up to his death at 73, while Ernst Mayr recently completed his monumental *The Growth of Biological Thought* (Harvard University Press, 1982) at 78. Musicians, of course, are famous for precocity but it is less appreciated that most great composers, except Sibelius, continued to be creative until felled by illness or death. Composers whose music unites words, voice and instruments particularly tend to peak very late in life — they are the evolutionary biologists of the musical world. Haydn, Janacek, Lalo and Rameau produced their greatest operas, oratorios, or masses between the ages of 65 and 72. Verdi's masterpieces *Othello* and *Falstaff* were completed at 74 and 80, while Richard Strauss's poignant and richly orchestrated

Erratum

In the fifth paragraph of Ian Stewart's article "How bent is a knot?" (14 March, p.130), the sentence beginning "If $n = 1$ (M is a curve)" should have continued "then $4\sigma_1$ must be replaced by $2\sigma_1$. And if $n = 3$, $N = 4$, then σ_1 is replaced by...".

Four Last Songs, looking back on his long happy marriage and its impending dissolution by death, were written at 84. By contrast, I am unaware of any Nobel Prize awarded for work done after the age of 65.

Do the changes in the creative style of composers with age offer a message for scientists aware of shifts in their own creativity? The desire for recognition often drives scientists cruelly to demand of themselves a time scale of creative development that their subject matter does not permit. It is not easy for young evolutionary biologists to be patient, nor older molecular biologists to be satisfied with an enforced change of intellectual goals as they age. Many composers confronted with similar problems were able to find inner peace. Richard Strauss, like his equally precocious librettist Hugo von Hofmannsthal, remained creative to the end, but was well aware of, and adapted to, changes in his abilities. No longer able to spin out the long musical phrases that had marked the symphonic poems of his youth, he turned instead to operas based on much shorter themes. The published correspondence of Hofmannsthal and Strauss[8] will fascinate scientists concerned with mastering the problems of publishing or perishing as they grow older. They might also bear in mind that as his total output (especially of sacred contatas) shrank with age, Bach concentrated on his two contrapuntal masterpieces, the *Musical Offering* and *Art of the Fugue*. □

1. Slonimsky, N. *Lexicon of Musical Invective*, 2nd edn (University of Washington, Seattle, 1975).
2. Nelson, G. & Rosen, D.E. (eds) *Vicariance Biogeography* (Columbia University, New York, 1980).
3. Dennis, W. *Science* **123**, 716 (1956).
4. Roe, A. *Science* **150**, 313 (1965).
5. Eiduson, B.T. in *Aging and Achievement* (ed. Taylor, C.W.) (Pennsylvania State University, University Park, 1968).
6. Eiduson, B.T. *J. Personality Assessment* **38**, 405 (1974).
7. Goulet, L.R. & Baltes, P.B. *Life-span Developmental Psychology* (Academic, New York, 1970).
8. *R. Strauss — H. von Hofmannsthal Briefwechsel* (eds Strauss, F. & Strauss, A.) (Atlantis, Zurich, 1952).

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Nuclear structure

Taken with a grain of salt

from Virginia A. Zakian

THE cytoplasm of eukaryotic cells is permeated by a highly-ordered subcellular skeleton of filaments and membranes. Even non-structural cytoplasmic components such as glycolytic enzymes are firmly positioned within this three-dimensional array. It would not, therefore, be surprising if the nucleus possessed an organizing skeleton required for the proper positioning of sequences important for replication, transcription, and chromosome integrity. Indeed the appearance of chromosomes at cell division as well as recent studies on polytene chromosomes in *Drosophila* interphase nuclei[1] provide cytological evidence for nuclear order. A variety of biochemical studies also support this view, including the isolation by Laemmli and co-workers of what they call the nuclear scaffold[2-5]. In a recent paper, this group has explored the nature of DNA sequences associated with the scaffold in *Drosophila* cells in interphase and asked whether these associations change as a function of gene transcription: their answer is provocative.

It is still a matter of controversy whether a nuclear substructure exists at all. Scepticism centres on the methods used to prepare material suitable for biochemical analysis: because there is no assay for structural integrity, it is difficult to prove that interactions found in intact nuclei are maintained during isolation. Of the different procedures developed to probe nuclear substructure, the isolation of the nuclear matrix is the most widely used. The matrix is defined as the largely proteinaceous structure which remains after isolated nuclei are extracted with non-ionic detergents and high-salt (2 M NaCl) and then digested with DNase[6]. Prior to the digestion, chromosomes are attached to the matrix at multiple sites such that the DNA is organized into topologically-independent, supercoiled domains[7]. After DNase digestion, the matrix retains the approximate shape of the nucleus and exhibits three major ultrastructural features: an outer lamina, a residual nucleolus, and a fibrillar network.

There is little consensus as to the nature of the DNA sequences involved in attachment to the matrix: some reports indicate that it is a random subset of the genome whereas others find an enrichment for repetitive sequences or specific genes. But a number of studies in a variety of systems suggest that the matrix provides a structural support for DNA replication, in that newly synthesized DNA is tightly associated with the matrix (see, for example, ref.7). Moreover, many workers find that structural genes are preferentially associated with the matrix in cell types in which the gene is actively transcribed (see, for example, ref.8). For some investigators these data are sufficient to indicate that the matrix serves a crucial role in organizing nuclear events; for others, they are artefacts of isolation, in that exposure of nuclei to high salt could induce precipitation of proteins or transcription complexes onto the matrix as well as allow sliding of DNA attachment sites.

Although the relationship between the nuclear matrix and Laemmli's nuclear scaffold is not clear, they share certain structural properties: for example, a subset of nuclear proteins is common to both and both include the peripheral nuclear lamina. The scaffold is prepared from the metaphase chromosomes of mammalian cells by the depletion of their histones and most other nuclear proteins, leaving a proteinaceous axial structure which retains the approximate shape of the original chromosome and is surrounded by a halo of DNA loops[2]. A rapidly-sedimenting structure with similar biochemical properties has been isolated from interphase cells[3]. The scaffold from both mitotic and interphase cells is sensitive to disruption by chelation and is believed to be held together by metalloprotein interactions[4,5].

For their recent investigation of the scaffold-associated DNA sequences in *Drosophila* interphase cells, Laemmli's group has developed a gentle, low-salt procedure of nuclear lysis. Isolated nuclei are extracted with buffers containing spermidine and lithium di-iodosalicylate (LIS), a salt with detergent-like properties which can be efficiently removed after use. These histone-depleted nuclei are washed, pelleted, and digested with restriction enzymes which solubilize about 70 per cent of the DNA. Both the solubilized DNA and the DNA in the residual nuclear scaffold are then assayed for the presence of specific sequences, including fragments of histone and heat-shock genes.

When fragments derived from the five-kilobase histone gene repeat unit are examined, one fragment is found exclusively with the scaffold whereas all the other fragments are found primarily in the supernatant. The scaffold-bound fragment is rich in adenine and thymine (A + T) and is derived from a non-transcribed 'spacer' sequence upstream of the histone 1 gene. Likewise an A + T-rich fragment from the non-transcribed spacer of the *hsp70* heat-shock genes is scaffold-associated. Moreover, this association is independent of the transcriptional state of the *hsp70* genes. Finally, many copies of a middle-repetitive A + T-rich element, including a copy found upstream of the *hsp70* gene, are scaffold-bound. These results suggest that the DNA loops of interphase nuclei are maintained through sequence-specific associations with scaffold proteins and that these associations do not change as a function of transcriptional activity. None of the specific associations are maintained if LIS-extracted nuclei are treated with 2 M NaCl after digestion with restriction enzymes. One interpretation is that a high concentration of salt disrupts the specific DNA-protein interactions in the scaffold; at the

1. Mathog, D., Hochstrasser, M., Gruenbaum, Y., Saumweber H. & Sedet, J. *Nature* **308**, 414 (1984).
2. Paulson, J.R. & Laemmli, U.K. *Cell* **12**, 817 (1977).
3. Lebkowski, J.S. & Laemmli, U.K. *J. mol. Biol.* **156**, 309 (1982).
4. Lewis, C.D. & Laemmli, U.K. *Cell* **29**, 171 (1982).
5. Mirkovitch, J., Mirault, M-E. & Laemmli, U.K. *Cell* **39**, 223 (1984).
6. Berezney, R. & Coffey, D.S. *J. Cell Biol* **73**, 616 (1977).
7. Vogelstein, B., Pardoll, D.M. & Coffey, D.S. *Cell* **22**, 79 (1980).
8. Ciejek, E.M., Tsai, M-J. & O'Malley, B.W. *Nature* **306**, 607 (1983).

same time it induces non-specific associations of active-gene regions through precipitation of transcription complexes[5].

These results are provocative: although they support the hypothesis that the nucleus is highly ordered, they raise doubts about the validity of methods widely used to analyse nuclear structure. However, in the absence of an accepted assay for structural integrity, this report must be evaluated with caution. It is possible that the LIS-extraction procedure introduces its own artefacts: for example, it might induce precipitation of A + T-rich sequences on to the residual scaffold. It is also possible that there are different classes of DNA-protein associations in intact nuclei, only some of which are transcription-dependent and only some of which are maintained through different isolation procedures. For example, it is easy to imagine that DNA interactions with the peripheral lamina will display a different sensitivity to extraction conditions than do interactions with the internal fibrillar network.

The argument against high-salt methods for the study of nuclear structure will be strengthened if others obtain similar results in different systems. Convincing proof of the importance of a given nuclear substructure may ultimately require the identification of mutations in its components; investigations in a lower eukaryote, amenable to genetic analysis, may be the most profitable way to establish functional basis for nuclear structure. □

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Magnetochemistry

How many atoms make a metal?

from D. Michael P. Mingos

MAN'S ability to recognize and exploit the characteristic properties of metals has been an important part of his technological development, although the magnetic and electrical properties of metals have only been fully utilized in the past hundred years. The scientific interpretation of these characteristics is based on a band-theory model, which raises the question of whether the characteristic properties of a metal are still displayed by the molecular clusters containing as few as 10 - 100 metal atoms that have been synthesized in recent years. The paper by D.C. Johnson *et al.* on page 231 of this issue examines transition metal cluster compounds and suggests that certain magnetic properties that they are characteristic of the bulk metal begin to appear when as few as ten metal atoms are present.

The band-theory model describes a metal as an infinite and regular three-dimensional array of atoms where electrons are no longer localized but are shared between the constituents, which are thus able to respond cooperatively to external forces. Ideally, studies on emergent metallic properties should be carried out on small-diameter particles (5 - 100Å) but these pose severe experimental and technical problems. First, such particles do not all contain the same number of metal atoms but have a statistical distribution of sizes. Second, to avoid contamination by oxide species, it is necessary to study them under high vacuum conditions.

The strategy adopted by Johnson and his colleagues avoids these difficulties by investigating molecular cluster compounds of osmium, composed of 3 - 10 atoms, that are held together at distances comparable to those found in the bulk metal. These clusters have a small chunk of bulk metal at their centre, but each metal atom is also bonded to carbon monoxide molecules, which stabilize the cluster and provide a protective sheath to stop the cluster aggregating into particles of bulk metal. Such cluster compounds have been synthesized in large numbers in recent years, particularly by research groups in Cambridge and Milan, and have been structurally characterized by single-crystal X-ray techniques. As a result, well-characterized compounds with fixed numbers of metal atoms and high purity are now available in reasonable quantities. They are generally air-stable and highly-coloured crystalline solids.

Johnson *et al.* have measured the magnetic properties of these compounds using a high sensitivity computer-interfaced Faraday magnetic balance in the temperature range 1.5 - 300 K. The compounds show a temperature-independent paramagnetic susceptibility, which is not surprising since, in a formal sense, all the electrons are paired up in metal-metal bonds. The observed paramagnetism arises from excited states that are not thermally accessible. The interesting aspect of the results arises from a positive correlation between the observed paramagnetism and the number of metal atoms in the cluster. They argue that the paramagnetism increases in this sequential fashion because molecular electronic states begin to develop into the band and continuum states that are characteristic of the bulk metal. The number and energies of the excited states increase with the number of metal atoms in the cluster, as demonstrated by a red shift in the electronic spectra, and consequently they make a larger contribution to the observed paramagnetism. This leads them to predict that transition-metal cluster compounds containing more metal atoms may show very interesting electronic properties that arise from a polarization catastrophe within the clusters themselves.

The authors are more equivocal in their answer to the fundamental question of how many atoms are needed in a cluster to mimic effectively the bulk properties of a metal. This depends on the particular property being measured. Transport properties, such as electrical conductivity, require complete ionization of electrons from the parent site — a process which might require 0.1 - 10 eV. — before metallic character can be detected. Magnetic properties, in contrast, are observed for clusters with quite small numbers of metal atoms, because modification of the intra- and inter-unit exchange energies requires only about 10^{-3} eV.

An industrial chemist is more likely to want to know how big the cluster need be before it performs effectively and selectively as a catalyst in a chemical reaction, a question that cannot be answered by this type of magnetic measurement. Chemists have, however, now synthesized molecular clusters with up to forty metal atoms, so no doubt they will soon be able to design suitable experiments to answer this question. □

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Satellite oceanography

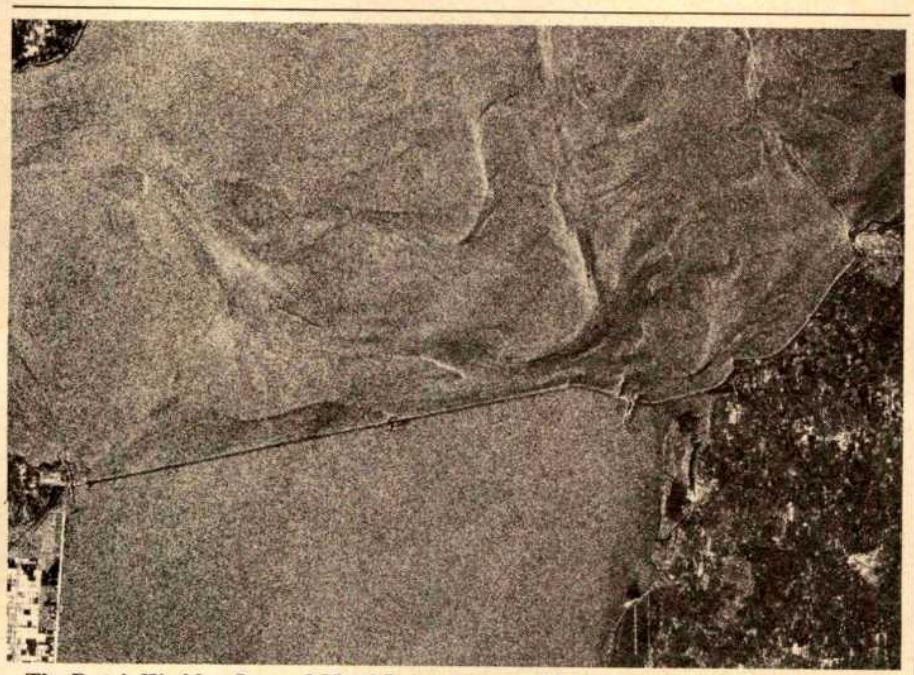
Radar images of the sea bed

from J.F.T. Zimmerman

SINCE G.P. De Loor first observed sand-wave-like features in radar images of the sea surface[1,2], the SEASAT satellite has produced many examples of the radar imaging of bottom topographic features[3,4]—as in the accompanying figure. Whether taken from side-looking airborne real-aperture radar (RAR)[1,2] or satellite-borne synthetic radar (SAR)[3,4], at first sight a sea bottom topographic image is something of a mystery. In a recent paper[5] (see also page 245 of this issue[6]), Alpers and Hennings have given a firm theoretical foundation to the most likely solution of the mystery.

The images may seem to be mysterious for two reasons. First, radar waves do not penetrate into the sea for more than a centimetre, so no direct reflectance from the bottom is possible in waters which may be up to some decametres in depth. Second, since radar backscattering from the sea surface occurs primarily by first-order Bragg-scattering[7] (on account of short surface

windwaves with a wavelength half that of the horizontal component of the radar wavelength — of the order of several centimetres to decimetres) and since, as a rule of thumb, surface waves do not 'feel' the bottom when the water depth exceeds half the wavelength, the Bragg-resonant surface waves cannot form an image of the bottom topography in any direct way. So, from the outset it has been clear that it is necessary to find at least one extra link, connecting modulations in the amplitude of the scattering surface waves to fluctuations in water depth. Although several hypotheses have been raised for this missing link, one has always been a strong candidate. It invokes the modulation of the (tidal) current velocity field by the bottom topography, the resulting spatial variability of the current velocity field in turn modulating the energy density of the relevant part of the surface wave spectrum[8]. It is this mechanism that has now been given a firm theoretical basis by Alpers and Hennings[5].



The Dutch Wadden Sea and IJssel Lake, separated by an enclosure dyke, as viewed by SEASAT-SAR. Whereas the Wadden Sea has strong tidal currents, the dyke excludes them from the IJssel Lake. The tidal channel system in the Wadden Sea is clearly visible but the relict tidal topography in the IJssel Lake is not visible due to the absence of tidal currents. (Courtesy National Aerospace Laboratory, Amsterdam).

Their contribution arises from a synthesis of theoretical results aleady obtained for the various interactions between electromagnetic (radar) waves, surface waves, tidal currents and bottom topography. For the modulation of tidal currents by the sea bed, the simplest possible approach is followed, resting on two assumptions. One is that there is divergence-free volume transport, which gives an inverse relationship between the cross-isobath velocity component and the water depth; the other is that the along-isobath velocity component is independent of position. Although the latter may be an oversimplification in the light of velocity-current interaction theories[9,10], the former is certainly justified for topographic length scales that are small compared with the tidal wavelength. Thus any tidal current with a velocity component in the direction of the local bottom slope produces divergences or convergences in the velocity field. As Alpers and Hennings show, these velocity gradients can produce images of the slope of the sea bed (rather than the depth itself) in two ways, one of which applies only to SAR and the other to both SAR and RAR.

The first mechanism is, in fact, an artefact of the SAR imaging process, called 'velocity bunching'. In the absence of currents, the azimuthal (along-flight) position of a SAR target is determined by the vanishing of the Doppler frequency shift when the target is at a right angle to the flight direction. When the target moves with a current having a component in the radial direction of the radar beam, an additional Doppler shift is produced so that the target is given a false position in the image in the azimuthal direction. In the presence of gradients in the radial velocity, targets are differently misplaced causing the otherwise homogeneous grey tone to show areas of dark and light colouring according to the sign of the velocity gradient, which in turn is related to the bottom slope.

Alpers and Hennings estimate the strength of this process to be nearly an order of magnitude weaker than the second mechanism, which involves the hydrodynamic interaction between the Bragg-resonant surface waves and the tidal velocity field. Two ingredients of surface-wave theory[11] are relevant to this mechanism. Waves propagating at the sea surface in a nonhomogeneous horizontal current-velocity field experience both refractive effects and an energy exchange with the current-velocity field due to the wave-radiation stress working against the strain-rate of the velocity field. This causes the wave energy density to increase when the current velocity increases in the direction of propagation, and *vice versa*.

This, in itself, would provide a mechanism of imaging but a second process also plays an important part. The short Bragg-resonant surface waves are located in that part of the wavenumber spectrum that is called 'saturation range'. This denotes the quasi-steady state in which a delicate, but poorly understood, balance between wind-forcing, nonlinear interaction among different wave components and dissipation by wave breaking maintains a spectrum in which the energy density falls with the fourth power of the wave number. So, if the straining of the velocity field perturbs the energy level of a certain wave component in the saturation range, one may expect this component to relax to its original value either by enhanced breaking (in the case of oversaturation) or by feeding upon the wind (in the case of undersaturation). Alpers and Hennings choose to parameterize this process by simple linear relaxation, characterized by a relaxation time scale that is of the order of a minute at most, and is short compared with the time (of the order of 5–60 minutes) that the waves need to travel over a typical bottom surface. The perturbation in energy density level for a specific location is given by a simple balance between the forcing due to the strain-rate of the velocity field and the relaxation process, and so it is proportional to the velocity gradient. This, in turn, is proportional to the bottom slope (divided by the square of the depth), so the energy density of the scattered waves — and thus the intensity of the radar signal — is largest on the downstream side of the ridge and smallest on the upstream side. As a consequence, when the current flows in opposite directions during the ebb and flood of the tide, a sign reversal of the image intensity should occur, which is indeed observed in RAR images[1,2]. This, together with the estimated relative strength of image-contrasts (20–30%), represents a firm corroboration of the theory.

Evidently, the theory also applies to the imaging of any other phenomenon that causes velocity divergences or convergences at the ocean surface — for instance, internal waves, trapped at strong vertical density gradients in the ocean interior, produce an undetectable vertical displacement at the ocean surface. Such waves have shown up in radar images and again can be explained by the stretched formalism[6].

Successful as the theory may look, it rests on some drastic simplifications, in particular on the form and strength of the relaxation mechanism. This is clearly brought out by comparing the paper of Alpers and Hennings with that of Phillips[12], which runs more or less along the same lines, albeit without a discussion

of topography-current interactions. The basic difference is that Phillips uses a more sophisticated parameterization of the relaxation. He stresses the fact that return to equilibrium by wave breaking in the case of oversaturation can be a more vigorous process than feeding upon the wind in the case of undersaturation. This may lead to strong asymmetry in the imaging process even in cases where the imaged current-velocity field has a symmetrical shape itself. Under certain conditions this leads to remarkable deviations from the results of Alpers and Hennings. For instance, when a local divergence of the velocity field decreases the wave-energy density, return to equilibrium may be so slow that the actual balance is not between straining and relaxation but between straining and advection. In this case, it is not the gradient of the current but the current velocity itself that is imaged by the radar (not the slope of the bottom but its inverse depth). For convergence, on the other hand, the relaxation of oversaturation by wave breaking may happen so quickly that excessively large strain rates are necessary to produce sufficient image contrast.

Although observations seem to support Alpers and Hennings, it is no surprise that careful observations of the relevant physical parameters in tidal areas are recommended in both papers to settle the dispute over the relaxation time scale. Perhaps this is a pious wish — but both off the Dutch coast in the southern North Sea and at the Nantucket shoals near Massachusetts[13], experiments have been set up to tackle these questions. □

1. De Loor, G.P. & Brunsveld van Hulten, H.W. *Boundary-layer Meth.* **13**, 119 (1978).
2. De Loor, G.P. IEEE *J. Oceanic Engng.* OE-6, 124 (1981).
3. Lodge, D.W.S. in *Satellite Microwave Remote Sensing* (ed. Allan, T.D.) 246 (Ellis Harwood, Chichester, 1983).
4. Kenyon, N.H. in *Satellite Microwave Remote Sensing* (ed. Allan, T.D.) 261 (Ellis Harwood, Chichester, 1983).
5. Alpers, W. & Hennings, I. *J. geophys. Res.* **89**, 10529 (1984).
6. Alpers, W. *Nature* **314**, 245 (1985).
7. Valenzuela, G.R. *Boundary-layer Meth.* **13**, 61 (1978).
8. Valenzuela, G.R., Chen, D.T., Garrett, W.D. & Kaiser, J.A.C. *Nature* **303**, 687 (1983).
9. Huthnance, J.M. *Estuar. coast. mar. Sci.* **1**, 89 (1973).
10. Zimmerman, J.T.F. *J.mar. Res.* **38**, 601 (1980).
11. Phillips, O.M. *The Dynamics of the Upper Ocean* 2nd edn (Cambridge University Press, 1977).
12. Phillips, O.M. *J. phys. Oceanogr.* **14**, 1425 (1984).
13. Valenzuela, G.R., Chen, D.T., Garrett, W.D. & Kaiser, J.A.C. *EOS Trans. Am. Geophys. Un.* **64**, 618 (1983).

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Blood volume regulation

On the right lines at last?

from W.E. Balfour

A WELCOME and secure step in our understanding of body fluid regulation is reported by Lang and his colleagues from Heidelberg on page 264 of this issue[1]. Their experiments on the rat heart show that a peptide is released from the atria in response to stretching in the isolated heart, or to expansion of blood volume in the intact animal. Since the peptide is one of the recently characterized atrial natriuretic factors that regulate fluid excretion by the kidney, the fluid regulation control loop between heart and kidney is confirmed.

The idea that blood volume is hormonally controlled is not new[2], nor is the possibility that low pressure elements of the heart can sense the fullness of the circulation[3]. What is new is that the heart atria contain secretory granules, the contents of which are released in response to stretch rather than depending on an automatic reflex. Lang *et al.* have shown these effects by using a radioimmunoassay developed after the atrial peptides had been isolated and synthesized[4]. The atria contain an immunoreactive molecule which is larger than that secreted in response to a stretch. Lang's group stretched the atria in two ways: by small increases in perfusion pressure in the vena cava of the isolated heart, and by expanding the blood volume by injection of graded quantities of saline in the intact animal. In each case, the response is a graded release of relatively large amounts of atrial peptide, which has a short half-life.

Unfortunately, the renal response was not measured by Lang *et al.* There are, however, two groups of experiments which do include a renal response; they are focussed on the left rather than the right atrium, but few would dispute that blood volume expansion raises the pressure in both atria. Linden's[5] experiments dissect the functional components of the response to distension of the left atrium, brought about by inflation of balloons, in anaesthetized dogs. The renal response, requiring an intact vagus nerve, is an increase in water excretion, which, in the short term of these experiments, does not result in any substantial correction of the plasma volume, the expansion of which is simulated by the balloon. Ledsome[6] has recorded similar reversible changes together with a fall in plasma vasopressin concentration, a result which is consistent with the hypothesis that atrial distension leads to a water diuresis.

On the othe hand, Kaczmarczyk *et al.*[7], working with surgically-prepared, yet conscious, trained and confident dogs, have used three stimuli to raise the atrial pressure and thus to effect a brisk increase in the excretion of both salt and water. Two of the stimuli, intravenous saline loading and ingestion of a sodium-containing meal, are just as effective after denervation of the heart, whereas stimulation by mitral stenosis, which raises only the left atrial pressure, is not effective after denervation. It is of particular interest that all of these effective stimuli are associated with a fall in the concentration of plasma renin. Only the stimulus of mitral stenosis is prevented by denervation of the heart.

More recently, Lee *et al.*[8], also working with conscious dogs, have shown that partial constriction of the ascending aorta fails to provoke the release of renin, even though the downstream fall in arterial pressure is similar to that which would be effective if the descending aorta were constricted. Constriction of the ascending aorta raises the left atrial pressure which inhibits renin release, probably through a hormonal mechanism. Thus, in these manoeuvres, increasing atrial stretch reduces the plasma concentrations of both renin and, it seems, vasopressin. The crucial question is whether these changes are causally related to the renal elimination of sodium and water in the proportions required for volume correction.

There have been few studies describing how atrial natriuretic factor causes natriuresis and diuresis. The reported potency of the peptide varies widely. This is probably related to whether the animals are conscious or anaesthetized, hydrated or dehydrated, sodium deficient or replete. Much less material is required if the renal artery is the route of administration but, so far, only anaesthetized animals have been used.

Two renal effects of atrial natriuretic factors — vascular and renin-reducing — have so far been recognized. The first is a specific reduction in renal vascular resistance[9], associated with which is an increase in renal papillary plasma flow[10] as high as that observed in rats congenitally lacking vasopressin (Brattleboro rats)[11]. Such increases in renal medullary plasma flow would be expected to dissipate (as they do in Brattleboro rats) the medullary osmotic gradient, which is a prerequisite for the excretion of a copious isotonic urine. Brattleboro rats, or rats whose blood vasopressin concentration is low from having recenty drunk water, excrete an isotonic urine at rates as high as one ml min^{-1} in response to maintained blood volume expansion[12].

Urine flows of one ml min^{-1} represent such a large recovery of the glomerular filtrate that they can be achieved only at the expense of a reduction in the normal level of fluid reabsorption in the proximal tubule. It is possible that the second action of atrial natriuretic factor — reducing renin secretion — is important here. Proximal-tubule puncture studies in rats whose renin-generated angiotensin II levels have been reduced by captopril, show a marked reduction in sodium reabsorption[13], and Shirley[14] has argued that the increased sodium excretion seen on captopril treatment of anaesthestized Brattleboro rats is likely to be a proximal-tubule effect.

The distal tubule has a much smaller role to play in the control of natriuresis,

particularly in short-term volume control, although it can become important as a fine control mechanism in the longer term. It is interesting that the angiotensin II-stimulated release of aldosterone from adrenal glomerulosa cells[15-17] can be inhibited by physiological amounts of the atrial factor and that avid receptors for the atrial peptide have been found in kidney tubules in smooth muscle of the vasculature and in glomerulosa cells[18].

It is necessary to ask whether the fall of both plasma renin and of vasopression exerts a regulatory or permissive role on the excretion of salt and water. This will not be answered until there have been measurements matching the observed changes in the concentration of atrial factor in the plasma to the different sensitivities in the control of the secretion of these two hormones. There is a long way to go before we can emulate Verney's observation[19], in the allied field of water balance, that a one per cent rise in the osmotic pressure of blood going to the brain is sufficient to produce a maximal antidiuresis. □

1. Lang, R.E. *et al. Nature* **314**, 264 (1985).
2. de Wardener, H.E. *Clin. Sci. mol. Med.* **53**, 1 (1977).
3. Henry, J.P. *et al. Circ. Res.* **4**, 85 (1956).
4. Atlas, S.A. *et al. Nature* **309**, 717 (1984).
5. Linden, R.J. & Kappagoda, C.T. *Atrial Receptors* (Cambridge University Press, 1982).
6. Ledsome, J.R. *et al. J. Physiol. Lond.* **338**, 413, (1983).
7. Kaczmarczyk, G. *et al. Pflügers Arch.* **390**, 125 (1981).
8. Lee, M.E. *et al. Fed. Proc.* **43**, 995 (1984).
9. Oshima, T. *et al. Circ. Res.* **54**, 612 (1984).
10. Borenstein, H.B. *et al. J. Physiol. Lond.* **334**, 133 (1983).
11. Bayle, F. *et al. Pflügers Arch.* **394**, 211 (1982).
12. Balfour, W.E. *et al. J. Physiol Lond.* **332**, 94P (1982).
13. Harris, P.J. *et al. J. Physiol. Lond.* **327**, 93P (1982).
14. Shirley, D.G. *et al. J. Physiol. Lond.* **346**, 107P (1984).
15. Kudo, T. *et al. Nature* **312**, 756 (1984).
16. Chartier, L. *et al. Endocrinology* **115**, 2026 (1984).
17. DeLean, A. *et al. Endocrinology* **115**, 1636 (1984).
18. Napier, M.A. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 5941 (1984).
19. Verney, E.B. *Proc. R. Soc.* B135, 25 (1947).

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Solid state dynamics

Unsolved problems of creep

from J. Weertman

ON page 255 of this issue Doake and Wolff[1] bring new information to a very intriguing, unresolved problem in high temperature creep of crystalline solids. The outcome of their examination of creep flow of ice in polar ice sheets is of importance not only to glaciers but to the deformation of the Earth's mantle and, of course, to the understanding of the creep process itself.

The origin of the problem is the old observation by Harper and Dorn[2] of a creep rate that is proportional to the applied stress (Newtonian creep) in aluminium at relatively low stresses, in conditions such that the creep should be produced by dislocation motion and so is expected to be proportional to a power of the applied stress. This expectation arises from the fact that creep rate is proportional to the product of the dislocation density and the average dislocation velocity. The former quantity, in steady state, is proportional to the stress squared and the latter to the stress. Hence the creep rate is proportional to the cube power of the stress. (Higher powers are also possible[3,4].)

It was easy to dismiss the original Harper-Dorn experiment by claiming that steady-state creep had not been attained. The initial dislocation density in material that shows decelerating creep before steady-state creep is reached is higher than when steady-state conditions prevail. If the creep rate is measured only in a small initial strain region, the dislocation density may be approximately a constant during the course of creep experiments. Hence the creep rate falsely seems to be proportional to stress. Very recently, Soliman and Mohamed[5] have shown experimentally that this applies to the original Harper-Dorn experiment and to more recent experiments that were not extended to large strains. It does not, however, apply to experiments in which Harper-Dorn creep has been measured in metals with large strains[6].

Blacic and I[7] recently offered an explanation of Harper-Dorn creep that has yet to be tested experimentally. We pointed out that the temperature cycling that is inevitable in the usual high-temperature creep tests is sufficient to produce large chemical stresses on the dislocations by periodically altering the value of the equilibrium-point defect density. Hence the dislocation density can be controlled by the chemical stress rather than by the applied mechanical stress. The average dislocation velocity remains proportional to the applied mechanical stress and the creep is Newtonian. This explanation, that Harper-Dorn creep is an artefact of temperature cycling, does not work for Harper-Dorn creep of ice in glaciers and in polar ice sheets, because seasonal temperature fluctuations are effectively removed at depths greater than about ten metres. (This has also been pointed out by Lliboutry and Duval[8].)

Nor, for the same reason, does it work for Newtonian creep in the Earth's mantle. Glacial rebound data and the anomalous motion of satellites orbiting the Earth are very nicely accounted for by analyses of mantle deformation that are based on Newtonian creep for the mantle[9,10]. Moreover, Harper-Dorn creep has been observed by Poirier *et al.*[11,12] in a mineral analogous to one of the presumed major minerals of the lower mantle.

If Harper-Dorn creep is real, and not just a peculiar experimental artefact, life is made easier for modellers of both mantle flow and glaciers. They can use the simpler linear constitutive law in their work. But the rub is that transient creep still cannot be ruled out as the explanation of the evidence for mantle and glacier Harper-Dorn creep. The creep strains that are involved in the analysis of both glacial rebound and anomalous satellite motion are generally quite small. Hence it is still possible to dismiss the application of these results to convection currents in the mantle, where clearly the strains are quite large.

As pointed out by Doake and Wolff[1], the measurements they analysed may also involve non-steady-state creep. The tilt of boreholes gives the creep rate as a function of depth, but the shear stress also increases with depth. To have steady-state creep, the stress must remain constant. Their results can be accounted for either if ice at moderate stresses is indeed Newtonian or if transient creep effects make it appear to be Newtonian although it is not. The same is true of the evidence that indicates Newtonian mantle creep. To help resolve this problem, it would be well worth obtaining the spreading rate of the Ward Hunt Ice Shelf in the Canadian Arctic. This ice shelf is much thinner than the Antarctic shelves considered by Doake and Wolff, and should give another, large creep-strain datum point at a much lower stress level. If ice is Newtonian and if it can be shown that temperature cycles do not account for Harper-Dorn creep in laboratory experiments, the very interesting phenomenon of creep in crystalline solids will remain to be solved. □

1. Doake, C.S.M. & Wolff, E.W. *Nature* **314**, 255 (1985).
2. Dorn, J.E. & Harper, J.G. *Acta metall.* **5**, 654 (1957).
3. Weertman, J. in *Proc. 2nd. int. Conf. Creep and Fracture of Engng. Mater. and Struct.* (eds Wilshire, B. & Owen, D.R.J.) 1 (Pinreridge Press, Swansea, Wales, 1984).
4. Sherby, O.D. & Weertman, J. *Acta. metall.* **27**, 387 (1979).
5. Soliman, M.S. & Mohamed, F.A. *Mater. Sci. and Engng.* **68**, L23 (1985).
6. Mohamed, F.A. & Ginter, T.J. *Acta metall.* **30**, 1869 (1982).
7. Weertman, J. & Blacic, J. *Geophys. Res. Lett.* **11**, 117 (1984).
8. Lliboutry, L. & Duval, P. *Annales Geophysicae* (in the press).
9. Peltier, R. *Advances in Geophysics* **24**, 1 (1982).
10. Lambeck, K. *Nature News and Views* **309**, 584 (1984).
11. Cahn, R.W. *Nature News and Views* **308**, 493 (1984).
12. Poirier, J.P., Peyronneau, J., Gesland, J.Y. & Barbec, G. *Phys. Earth planet. Int.* **32**, 273 (1983).

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Scanning electron micrograph (× 975) of basalt glass after leaching in NaCl solution at 200°C for 30 days — a model for the stability of nuclear waste glasses. **See page 252.**

Protein engineering

Hydrogen bonds in active sites

from John Galloway

SINCE the sceptical reception of Latimer and Rodebush's suggestion in 1920 that they hold water molecules together, hydrogen bonds have come a long way. They are now widely believed to play the definitive role in determining molecular specificity. Hence the interest of protein engineers in hydrogen bonds and the paper on page 235 of this issue in which Alan Fersht and colleagues analyse, by means of systematically altering the amino acids of the active site of an enzyme, the hydrogen bonds that determine substrate specificity.

Full realization of the potential of protein engineering is some way off. The chief problem is our inexact understanding of the energetics of the three-dimensional structures of proteins and of the relationships between those structures and the proteins' functions. It is well enough established that the sequence of amino acids uniquely defines a protein's three-dimensional structure and, in doing so, confers on it a capacity to interact functionally with perhaps only one other molecule. Driven to the molecular level, the study of biological specificity thus becomes the detailed analysis of this structural and functional idiosyncracy and the mechanisms by which it is achieved.

Without a detailed understanding of the way proteins are put together, it is inconceivable that new proteins can be designed or old proteins given new roles. But that understanding is hard to win in the degree of detail needed, and the capacity to determine structure and function by protein crystallography still far outstrips any attempt to predict it from amino-acid sequence. It may become possible to mimic protein folding by analogue means but otherwise we are left with energy calculations which are not easy. Although, among other things, fair calculations can be done '*in vacuo*', proteins fold in water, which probably mediates all interactions of any interest. Since it is now possible to change any residue in a protein by site-directed mutagenesis of its gene, the present compromise is to study empirically the structural and energetic changes associated with small changes in amino-acid sequences.

For Fersht *et al.*, the protein under study is the enzyme tyrosyl-transfer RNA synthetase which activates tyrosine, forming a complex between the enzyme and the intermediate tyrosyl-adenylate, before transferring the amino acid to its transfer RNA. (The enzyme is of no apparent medical or commercial interest.) The power of the approach lies in combining site-directed mutagenesis both with enzyme kinetics, which gives accurate estimates of changes in binding energies, and with high-resolution crystallography, which gives the detailed structural changes. (Without high-resolution crystallography the technique can still be used, but merely as a probe of the role of residues in catalysis, for instance.) A logistical difficulty that such combined methodology usually has to face is that the techniques of mutagenesis and kinetics are quick to yield results, but protein crystallography tends to lag behind. It is striking that this is not the case for the tyrosyl-transfer RNA synthetase studies, presumably in part because of growing familiarity with its structure.

The most noteworthy of a number of findings described by Fersht *et al.* is that a hydrogen bond involving a side chain with a net charge on it is stronger — by up to a factor of about six — than a hydrogen bond with no such involvement. Indeed by the time the difference in energy is properly weighted by a Boltzman exponential factor, a 'charged' hydrogen bond may well contribute one to two orders of magnitude more to specificity than an 'uncharged' one. That is useful to know in building up a database of interactions and it also reminds us of something that is often forgotten — hydrogen bonds are to a large extent simply a convenient way of looking at a part of the overall spectrum of electrostatic interactions between molecules or parts of molecules. A convenient way of expressing such an interaction is in terms of a multipole expansion

$$\frac{e_1e_2}{r_{12}} + \frac{e_1\mu_2 + e_2\mu_1}{r_{12}^2} + \frac{\mu_1\mu_2}{r_{12}^3} + \cdots\cdots$$

where e represents net charge and μ a dipole and 1, 2 refer to the interacting groups. The leading term in computing a hydrogen bond between neutral groups — like water molecules — is the third one. But if one group has a net charge, then the second term will lead and typically will be a good deal bigger. Since the problem is complicated by the presence of strongly dipolar water molecules, it would not have been very surprising had the effect of the charged hydrogen bond not shown up. That it has done, is well worth noting.□

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Ecology

Competition in imaginary worlds

from Paul H. Harvey and Robert M. May

TO WHAT extent does competition influence the distribution of species among the islands of an archipelago? This seemingly straightforward question cannot easily be answered despite scores of studies using a variety of techniques to analyse the distributions of many plant and animal species from several groups of islands [1]. No consistent patterns emerge. Indeed, various authors working on the same data set often reach different conclusions because they have used different assumptions in their statistical models. A new approach by Colwell and Winkler [2],using a computer to generate a myriad of imaginary worlds, helps us to understand the limitations of conventional analyses for detecting the factors that actually govern the distribution of species in archipelagos.

The essential problem is that each archipelago is unique, with its plant and animal species put in place by the slow workings of evolutionary processes. The logically appealing techniques of The Scientific Method — manipulative experiments with controls and replicates — are not easily applied in the real world. As emphasized by Simberloff, Strong and others at Florida State University [3], one way of trying to tease out the processes underlying observed patterns of distributions of species is to compare these patterns with null models constructed by randomly reshuffling the species among the islands. But the effects of competition may still inadvertantly be built into the null models, which assume that competition is absent

Colwell and Winkler[2] shed light on these problems by generating imaginary archipelagos, each populated according to specified rules. Using the worlds they have created, they explore the extent to which conventional methods of analysis can detect the competitive or other factors that actually govern the distribution of species in their archipelagos.

They begin by generating phylogenies, using a simple simulation model designed by Raup and Gould[4]. As the hypothetical species evolve, their phenotypes change gradually so that, on average, closely related species are more like each other than are more distantly related species. For the purposes of the model, easily measurable characters such as beak length and width are chosen. This evolution takes place on a large land mass under the guidance of a computer program called GOD. The organisms then disperse to the islands of a nearby archipelago, their destinations determined by additional computer routines, summarized in the figure.

One routine allows random invasion of the different islands with subsequent interspecific competition determining which species establish successful populations on each island. When phenotypically similar species invade an

island, some are likely to become locally extinct as a result of competitive exclusion. An alternative routine allows random invasion of islands from mainland without subsequent competition. As competition occurs only in the first routine, the second makes a suitable null model for comparison. One recent empirical study uses exactly this method: Graves and Gotelli [5] compare the actual avifaunas on land-bridge islands in the New World against the distribution patterns generated by sampling appropriate pools of mainland species.

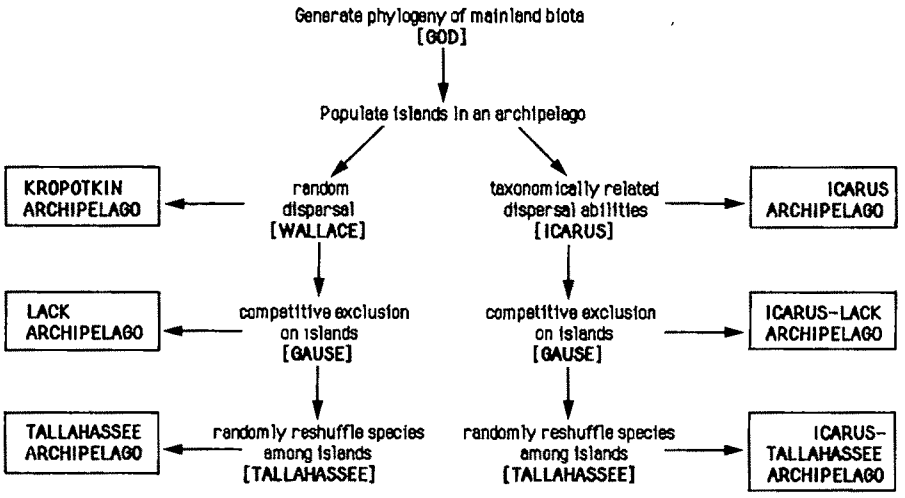
But a different type of comparison is often made with real data. Species found on different islands are randomly redistributed among the same islands (by computer of course); the reassortment is then compared with the original distribution pattern [3]. Colwell and Winkler incorporate a computer routine that mimics this procedure. They find that the species distributions differ very little between the reassorted archipelago and the one on which competition has occurred, thus confirming that the effects of competition are indeed partly incorporated into the null model derived from the reassortment routine. Consequently a comparison that uses this procedure is less likely to detect the effects of competition than is Colwell and Winkler's first procedure [6,7].

Another factor, usually ignored, that leads to underestimating the influence of competition, is the varied abilities of different taxa to disperse [6,8]. For example, kingfishers are more vagile than kiwis, and so are likely to be found on islands which kiwis have not reached. In a second series of comparisons, Colwell and Winkler allow dispersal abilities as well as morphological characteristics to evolve in the phylogenies generated by their computer. The result is that closely related species tend to end up on the same islands. Some species are extirpated when competition occurs, thus producing a species assemblage on each island that is taxonomically less biased than that of the original invaders. Because differential dispersal abilities produce the opposite pattern from competition if they are ignored when designing null models, the effects of competetion are shown to be consistently underestimated.

Colwell and Winkler have also contributed to the comparisons of the distributions of species that are not likely to be in competition, the subject of several recent discussions[9-11]. Classic cases in the literature include searching for competitive exclusion between owls and hummingbirds or between ducks and warblers [12]. The simulation study helps to place this problem in context. Since the computer has a complete phylogenetic record, it can assign relative taxonomic status with accuracy. Comparisons made at lower taxonomic levels, say among species within a genus, are far more effective for detecting the statistical consequences of competition — the level at which the process acts most strongly — than those that incorporate a wider taxonomic spread, say an order or a class. Here, however, the creator (GOD, or Colwell and Winkler) has a considerable advantage in knowing the likely relations between competitive intensity and the phylogenetic relationship among species, whereas such relations may be less clear to an observer in the real world. Colwell and Winkler could, if they wished, give objective and usable criteria for assigning subsets of species into "guilds" containing species with similar patterns of resource utilization, whereas such decisions about grouping real species usually involve some subjectivity.

The problems do not stop here. Sample sizes tend to be small and Type II statistical error (failure to reject an incorrect null hypothesis) tends to be high, making it very difficult to reject the null model from which the effects of competition have been excluded [2]. Even if it can be, there are often alternative interpretations of the patterns revealed [5]. For example, habitats differ between islands and the complimentarity of species distributions could result simply from differences in habitat preferences or realized niches [5,13].

In the nature of things, ecologists and biogeographers can often obtain no more than circumstantial evidence. On the one hand, the studies of Colwell and Winkler illustrate some of the difficulties and pitfalls in working with such circumstantial evidence. On the other hand, by seeking to design appropriate null hypotheses that allow real patterns to be distinguished from statistical artefacts, they have achieved a constructive step in the direction indicated by Simberloff, Strong and colleagues [3]. Where manipulative field experiments are possible, the answers are usually much less ambiguous. Some experiments of this kind — for some taxonomic groups but not for others — point to the importance of competition in structuring natural communities [14,15]. □



Colwell and Winkler [2] generate a computer phylogeny using a routine called GOD, and distribute species among the islands of an archipelago according to rules specified by either WALLACE or ICARUS. The species on each island are then subjected to competition using GAUSE, to produce archipelagos with new species compositions. Finally the species on the post-competition archipelagos are redistributed among the islands using TALLAHASSEE. Recent analyses of real data sets search for the effects of competition by comparing the equivalent of the LACK and ICARUS-LACK ARCHIPELAGOS with the TALLAHASSEE and ICARUS-TALLAHASSEE ARCHIPELAGOS respectively. These comparisons consistently underestimate the effects of competition [2]. More suitable nulls models against which to compare the LACK and ICARUS-LACK ARCHIPELAGOES are the KROPOTKIN and ICARUS ARCHIPELAGOS respectively.

1. Harvey, P.H Colwell, R K , Silvertown, J W & May, R M *Ann Rev ecol. Syst* **14**, 189 (1983)
2. Colwell, R K & Winkler, D W in *Ecological Communities Conceptual Issues and the Evidence* (eds Strong, D R., Simberloff, D., Abele, L.G & Thistle, A B) 344 (Princeton University Press, Princeton, 1984)
3. Strong, D.E., Simberloff, D , Abele, L.G & Thistle, A.B (eds) *Ecological Communities Conceptual Issues and the Evidence* (Princeton University Press, Princeton, 1984)
4. Raup, D.M & Gould, S J *Syst Zool.* **23**, 305 (1974)
5. Graves, G R & Gotelli, N.J *Oikos* **41**, 322 (1983)
6. Grant, P R & Abbott, I *Evolution* **34**, 332 (1980)
7. Case, T J & Siddell, R *Evolution* **37** 832 (1983)
8. Simberloff, D *Am. Nat* **112**, 713 (1978)
9. Alatalo, R *Ecology* **63**, 881 (1982).
10. Diamond, J.M & Gilpin, M E *Oecologia* **52**, 64 (1982).
11. Wright, S J. & Biehl, C C *Am Nat* **119**, 345 (1982)
12. Connor, E.F & Simberloff, D *Ecol Monogr* **48**, 219 (1978).
13. Gilpin, M E. & Diamond, J M *Oecologia* **52**, 75 (1982)
14. Schoener, T W *Am. Nat* **122**, 240 (1983)
15. Strong, D R , Lawton, J H & Southwood, T R E *Insects on Plants: Community Patterns and Mechanisms* (Blackwell, Oxford, 1984)

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100 years ago

THE Prince of Wales, President of the International Inventions Exhibition, has fixed Monday May 4, for the opening of the Exhibition. Rapid progress is being made in all branches connected with the Exhibition. The large space set apart for machinery in motion is already being filled, whilst preparations for receiving other exhibits are well forward, some of which have arrived at the building. The Aquarium Department is receiving considerable attention, and will form a very attractive feature. Lord Onslow has lately presented 1500 exceedingly fine carp to the Aquarium, and a large number of fish indigenous to the Canadian Lakes have also been received for exhibition in the tanks.

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Mechanisms for homologous recombination

SIR — Fink and Petes [1] argued that the difference between a 50% frequency of crossing-over associated with meiotic gene conversion at homologous loci, on one hand, and absence of crossing-over associated with events involving repeated sequences at different locations in the genome on the other hand, suggested the existence of more than one pathway in homologous recombination. Fincham [2] and Whitehouse [3] stressed that the frequency of crossing-over associated with meiotic gene conversion varies widely and can be much smaller than 50%. They pointed out that eukaryote recombination can occur by a single pathway that entails two steps with gene conversion occurring in the first step and reciprocal exchange in the second: variation of crossing-over association might reflect special conditions required for the second step. Nevertheless, but for different reasons than Fink and Petes, we think that recombination events occurring at homologous positions in fungi may arise via several mechanisms.

Genetic analysis in fungi shows that gene conversion can occur via distinct pathways. It strongly suggests meiotic heteroduplex DNA intermediates, involving either one of the two interacting duplexes [4]. These intermediates can be matured by mismatch correction [5]. In yeast, gene conversion can occur by the repair of double-strand gaps [6] and it has been suggested that this might be a major pathway for meiotic recombination [7]. This diversity in the origin of gene conversion is illustrated by current models [7-9].

The competing models for gene conversion all retain the idea of a crossed strand junction at the end of the conversion area. They predict that crossing-over arises by the resolution of the junction and therefore is associated with gene conversion. These models cannot explain simply the evidence gathered over years of genetic analysis of the crossing-over mechanism. In particular we argue that reciprocal exchanges can be spatially and temporally separated from gene conversion. In *Sordaria* cross-overs can be separated from a site of gene conversion by a reigon in which a marker shows normal mendelian segregation [10]. In yeast, mitotic gene conversion events which occur in G_1 are sometimes associated with cross-overs in G_2 [11]. Unified models predict that frequency of gene conversion for a central marker should be high in cells which are recombinant for the flanking markers, when the interval between the outside markers is less than the average conversion length. However, in *Ascobolus*, reciprocal recombination selected within gene *b2* was found to be rarely associated with gene conversion in the interval [12].

Bacteriophage λ encodes functions for two recombination systems and is a substrate for host-encoded recombination. Why should eukaryotic genetic recombination involving much larger genomes be more simple? Eukaryotes should have evolved different recombinational systems to deal with the different situations in their genomes and life histories where recombination is required.

As an alternative to continual tinkering to explain unpalatable facts, it seems worth considering schemes which invoke multiple mechanisms for recombination. We believe that the recognition of this possibility may simplify models and rejuvenate research in the whole field of recombination.

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1 Fink, G.P. & Petes, T D *Nature* 310, 728 (1984)
2 Fincham, J R S *Nature* 312, 107-108 (1984).
3 Whitehouse, H L.K *Nature* 312, 108 (1984)
4 Paquette, N & Rossignol, J-L *Molec gen Genet* 163, 313 (1978)
5 Hastings, P.J *et al Curr Genet* 2, 169 (1980).
6 Orr-Weaver, T L *et al. Proc natn Acad Sci U S A* 78, 6354 (1981)
7 Szostak, J W *et al Cell* 33, 25 (1983)
8 Holliday, R *Genet Res.* 5, 282 (1964).
9 Meselson, M S & Radding, C.M *Proc natn Acad Sci U S A* 72, 358 (1975).
10 Thervendirajah, K & Whitehouse, H L K. *Molec gen Genet* 190, 432 (1983)
11. Roman, H & Fabre, F *Proc natn. Acad Sci. U S A.* 80, 6912 (1983)
12 Rossignol, J-L., *et al. Cold Spring Harb Symp quant Biol* (in the press)

Null hypotheses and prediction

SIR — In the past decade or so a subtle misuse of null hypotheses has become almost standard in ecology, biogeography, functional morphology and theoretical palaeontology. This comes from an overly strict adherence to the narrow Neyman-Pearson view of statistics as hypothesis-testing. The alternative approach of R.A. Fisher, himself an evolutionary biologist as well as the founder of modern statistics, emphasizes estimation, causation and the expansion of knowledge. (I simplify a complex subject.)

The difficulty here comes when a null hypothesis is placed in a privileged position, to be accepted at least provisionally until disproved. What we really want to know is how well it is supported compared with other possibiliites, not all of which may be precise or even known. For this one can compare likelihoods, calculate confidence intervals, and use similar, even qualitative, approaches which treat all possibilities as reasonable *a priori*.

It may be that, because of simplicity, theoretical coherence, prior knowledge, or the like, one possibility is indeed initially preferable, or the initial likelihoods of hypotheses may be unequal. Then one can merely incorporate such knowledge of via Bayes's theorem, precisely or very roughly, without the complexities of bayesian statistics. But it is rarely the case that one hypothesis is sufficiently preferable *a priori* that it should just be accepted until disproved.

For instance, the absence of adaptation for a particular structure should not be assumed until adaptation has been shown (itself often a difficult endeavour), although the possibility must be kept in mind. There are various mechanisms for nonadaptive and even inadaptive evolution at any one level [1,2], but most involve natural selection, and thus adaptation in some other way. Theoretical coherence based on a wide span of background knowledge actually gives a greater initial likelihood to direct or indirect adaptation without making, by itself, a precise and testable hypothesis.

Prediction is commonly associated with Popper, although it has been fashionable even in natural history since at least Cuvier's [3] time, and it is commonly regarded as the only way to do science. Yet much of the evolutionary half of biology, including the basic discovery by both Darwin and Wallace, is justified deductively [4], like mathematics. Within the domain where this is the case, hypotheses themselves are superfluous although prediction may be added, as Darwin did, to give additional justification.

We must not let the simplicity of a cookbook methodology blind us to real diversity. As Harvey *et al.* [5] recently quoted in another context, from Tukey [6], "Far better an approximate answer to the *right* question, which is often vague, than an exact answer to the *wrong* question, which can always be made precise."

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1 Van Valen, L M. *Amer. Nat* 94, 305-308 (1960)
2 Gould, S.J & Lewontin, R C *Proc R. Soc Lond* B 205, 581-598 (1979)
3. Rudwick, M J.S *The Meaning of Fossils* (Macdonald, London, 1972).
4 Van Valen, L.M. in *Conceptual Issues in Ecology* (ed Saarinen, E) 323-343 (Reidel, Dordrecht, 1982).
5. Harvey, P.H., Colwell, R K , Silvertown, J W & May, R M *A Rev Ecol Syst* 14, 189-211 (1983)
6 Tukey, J W *Annls math Stat* 33,1-67 (1962).

Scientific Corespondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

Study of magnetism in osmium cluster compounds as molecular models for small metallic particles

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The magnetic susceptibilities of a series of cluster carbonyl compounds of osmium have been measured by a high-sensitivity Faraday method and the evolution of certain aspects of 'metallic' behaviour in the high-nuclearity clusters is revealed by a steady increase in the excess molecular susceptibility as the cluster size increases. We also consider here 'metallic' status in the cluster compounds of other transition elements.

THE physical and chemical properties of small metallic particles with diameters < 100 Å (refs 1-6) are interesting because of their importance in heterogeneous catalysis and their position as intermediaries between the atomic and (bulk) metallic regimes. It is often asked in what manner, and at what nuclearity, atomic and molecular properties admit 'metallic' behaviour[1,6-13]. This description involves, to first order, the neglect of inter-cluster interactions and deals primarily with the evolution of the electronic structure of isolated cluster states. Representative examples include ultra-fine dispersed metal catalysts[2-4], 'naked' metal clusters formed via the controlled aggregation of metal atoms in low-temperature matrices[14-16], and, the subject of the present investigation, inorganic cluster compounds of the transition elements[17]. The study of small metallic particles poses severe experimental problems because of unavoidable distributions of particle size within a sample and the necessity for high-vacuum conditions to avoid contamination with oxide species[1]. In contrast, carbonyl cluster compounds of, for example, Os, Rh and Pt, of generic formula $M_m(CO)_n^{Q-}$, with $Q \geq 0$, $n > m$, are well characterized molecular species, obtainable in samples of very high purity, and many are air-stable. The cluster carbonyls of these three elements have been shown by X-ray crystallography to exhibit many different geometries (Fig. 1), which are fragments of extended metallic lattices (hexagonal close-packed (h.c.p.); face-centred cubic (f.c.c.); body-centred cubic (b.c.c.)), or resemble other types of close packing (for example, polytetrahedral, icosahedral), theoretically favourable for particles containing a few dozen atoms[17-20].

The compounds in Fig. 1 represent metallic cluster fragments isolated from adjacent cluster units by inert sheaths of carbonyl ligands; these cluster units are fundamentally different from, for example, those within the ternary molybdenum chalcogenides (ref. 21 and references therein), often called Chevrel phases, $M_xMo_6X_8$ (where M represents a rare earth or other metallic element and X is S, Se or Te), in which the chalcogen atoms occupy bridging positions between the metal cluster units, and extensive inter-cluster interactions give rise to itinerant electron behaviour at ambient temperatures, and superconductivity below ~15 K.

The transition-metal cluster carbonyls, therefore, may be ideal systems for monitoring the genesis of the electronic structure of particulate transition metals. We report here a systematic study of magnetism in the cluster carbonyls of osmium.

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Magnetic measurements

The magnetic susceptibilities of the various Os cluster compounds were measured over the range 1.5-300 K using a high-sensitivity computer-interfaced Faraday balance, described elsewhere[22]. Samples were suspended on a 1-mm quartz rod from the arm of a Cahn RG electrobalance. The sample container (4.2 × 10 mm) was fashioned from high-purity Spectrosil quartz. An electromagnet (Eastern Scientific Instruments) supplied variable fields up to 11.8 kG. The magnetic field gradient was measured using $HgCo(SCN)_4$ as a standard and varied by < 2%. Temperature was controlled (±0.1 K) and monitored with a digital temperature controller (Oxford Instruments, DTC 2) using a Au/Fe versus chromel/P thermocouple. A calibrated silicon diode resistance thermometer was used to measure the sample temperature, independently of the controlling thermocouple, over the entire temperature range. The Cahn balance was operated on the 1-g scale, on which a sensitivity in ideal conditions of 0.0005 mg can be achieved; in normal operating conditions, the sensitivity was only ~0.001 mg and the reproducibility was ~0.001 mg. A typical error estimate in the measurement of magnetic susceptibility is $\pm 10 \times 10^{-6}$ e.m.u. mol^{-1} for $Os_3(CO)_{12}$ and $\pm 30 \times 10^{-6}$ e.m.u. mol^{-1} for the cluster $H_2Os_{10}C(CO)_{24}$. For each sample, magnetic measurements were taken at three values of the magnetic field, at 8.8, 10.5 and 11.8 kG. The total measured susceptibility values of the Os cluster compounds at room temperature, which are (overall) diamagnetic, range from $-170(\pm 10) \times 10^{-6}$ e.m.u. mol^{-1} for $Os_3(CO)_{12}$ to $-700(\pm 40) \times 10^{-6}$ e.m.u. mol^{-1} for $Os_{10}C(CO)_{24}I_2$ and have been corrected for the intrinsic magnetism of the sample container.

The experimental quantity of most direct interest is the excess molecular susceptibility, χ_{ems}, of the Os cluster compounds, which represents the difference between the magnetic susceptibility of the molecular complex (Fig. 1) and the sum of those species from which the complex is composed. The natural choice for these constituents is Os and H atoms, freely rotating CO molecules and, in the case of $H_2Os_{10}C(CO)_{24}$, an additional C atom (the interstitial C atom, Fig. 1). Reliable values for Os (-66×10^{-6} e.m.u. mol^{-1}), I (-52×10^{-6} e.m.u. mol^{-1}), C ($+5.0 \times 10^{-6}$ e.m.u. mol^{-1}) and H (-2.93×10^{-6} e.m.u. mol^{-1}) are given by König[23]. The measured magnetic susceptibility for gaseous CO is -9.8×10^{-6} e.m.u. mol^{-1}. The observed molar susceptibilities and derived excess molecular susceptibilities at 298 K and 11.8 kG for six cluster compounds of Os are given in Table 1 and these limiting high-temperature values are plotted as a function of cluster nuclearity in Fig. 2. Note that the

experimental excess molecular susceptibilities (χ_{ems}) are considerably larger than the intrinsic errors in our measurements. (For example, for the low-temperature susceptibility of $H_2Os_{10}C(CO)_{24}$ to arise from Os (IV) impurities, some 20% of the molecules in the sample would have to be Os(IV) complexes. No evidence of such complexes was found by infrared spectroscopy or X-ray diffraction.)

The magnetic susceptibilities of the entire series of Os clusters are truly temperature-independent down to 1.5 K, except for the decanuclear hydrido-cluster, $H_2Os_{10}C(CO)_{24}$, which shows a temperature-dependent paramagnetism at low temperature, approximating closely to a Curie-type behaviour.

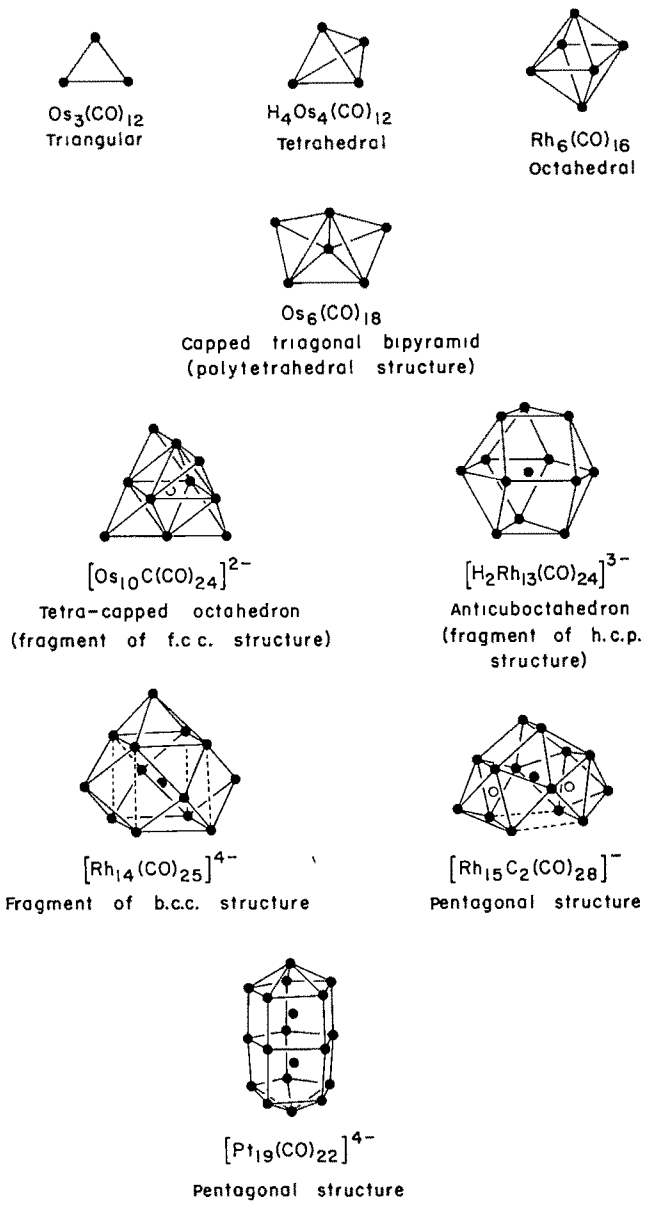


Fig. 1 A representation of typical metal atom geometries in transition metal carbonyls. For clarity, the carbonyl ligands have been omitted. Open circles denote interstitial carbon atoms in the cluster compound. **Materials.** The compounds $Os_3(CO)_{12}$, $H_2Os_3(CO)_{10}$, $H_4Os_4(CO)_{12}$, $Os_7(CO)_{21}$, $H_2Os_{10}C(CO)_{24}$ and $Os_{10}C(CO)_{24}I_2$ were prepared by existing methods[18,46]. The purity and composition of the samples were carefully checked by TLC, infrared spectroscopy and, in the case of the clusters containing <10 Os atoms, by mass spectrometry (AEI MS12, 100–150 °C). The elemental purity of all compounds was also tested by atomic absorption spectroscopy; the elements Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb and Pd were tested in a 47-mg sample of the decanuclear cluster $H_2Os_{10}C(CO)_{24}$ and were undetectable down to the limits of detection of the absorption spectrometer, which were 1 p.p.m. for Fe and Zn, and 0.1 p.p.m for the remaining elements.

Discussion

The excess molecular susceptibilities (Fig. 2), χ_{ems}, are most naturally interpreted as arising from a Van Vleck paramagnetic contribution to the susceptibility. Somewhat related magnetic behaviour has been observed previously in transition metal (molecular) complexes that have nominal singlet ground states, for example, in $Cr_2O_7^{2-}$, MnO_4^- and WO_4^{2-} complexes, as well as in Co (III) complexes possessing a low-lying excited state[24–26]. However, of particular importance here is the marked increase in χ_{ems} with increasing cluster size (Fig. 2) and how this relates to our view of the electronic structure of these materials.

In the present instance, a Van Vleck contribution[26] to the observed susceptibility will arise from modification of the cluster wavefunction by the external magnetic field. The modified wavefunction is described by the admixture of excited states in which electrons are promoted from delocalized bonding orbitals of the cluster to the delocalized antibonding orbitals. As the second-order perturbation expression for this paramagnetic contribution contains terms inversely proportional to the excitation energies between the occupied and unoccupied molecular orbitals[26,27], the increase in χ_{ems} with increasing cluster size suggests that the excitation energy gap decreases correspondingly. This interpretation (discussed below) would be invalidated if the matrix element $\langle n|\mu_z|0\rangle$, connecting the ground state $|0\rangle$ with the excited state $|n\rangle$ of energy $(E_n - E_0)$ above the ground state, changed significantly with cluster size. This, however, is improbable.

That relativistic effects, of which spin-orbit coupling is the best known, may well be significant in a heavy atom such as Os does not invalidate our qualitative interpretation of χ_{ems}. Although a closed-shell ground state will contain admixtures of states having non-zero total-spin, S, in relativistic theory, such a closed relativistic configuration will still be symmetric under time-reversal; the operator describing the interaction of the closed shell with the external magnetic field is antisymmetric and thus, under this operation, the first-order energy of interaction vanishes. Hence, the observed magnetic contribution consists, apart from the usual diamagnetic term, of second-order effects in which the wavefunction is modified by the magnetic field. Note that attempts to account for the delocalization of cluster bonding electrons in molecular orbitals would lead to larger diamagnetic terms, as the electrons would necessarily have larger effective radii than those confined to single Os atoms; and the values of the excess molecular susceptibility would then be increased with respect to those given in Table 1.

Several limiting descriptions are applicable to this Van Vleck temperature-independent paramagnetism (TIP), sometimes called the 'high-frequency' or 'second-order Zeeman' paramagnetism (see ref. 27 for a detailed review of the four important situations). Note that the same energy denominator that governs the magnitude of the Van Vleck paramagnetism is also responsible for the important paramagnetic contribution to the chemical shielding constant in nuclear magnetic resonance (NMR)[28]. However, one of the matrix elements in the NMR situation differs slightly from that in the Van Vleck paramagnetism.

In the normal case where the characteristic energy gap $(E_n - E_0)$, separating occupied from vacant orbitals, is large compared with the thermal energy, that is, $(E_n - E_0) \gg kT$, the induced paramagnetism is truly temperature independent[27,29]. The Os clusters would, at first sight, be expected to have a pure spin-singlet magnetic ground state. Thus, theories of bonding have successfully concentrated on the number of skeletal electron pairs required to bind the metal cluster unit, implicitly assuming a pure diamagnetic ground state[30,31]. The magnetic behaviour (Fig. 2) suggests that this picture is reasonable for small clusters, but becomes inappropriate for larger clusters in which there is obviously a large reduction in the frontier-orbital energy gap. Overall, then, we attribute the increasing Van Vleck TIP contribution for the high-nuclearity clusters primarily to a reduction in $E_n - E_0$, identified here as a measure of the separation of the frontier orbitals in the cluster[32]. A theoretical molecular

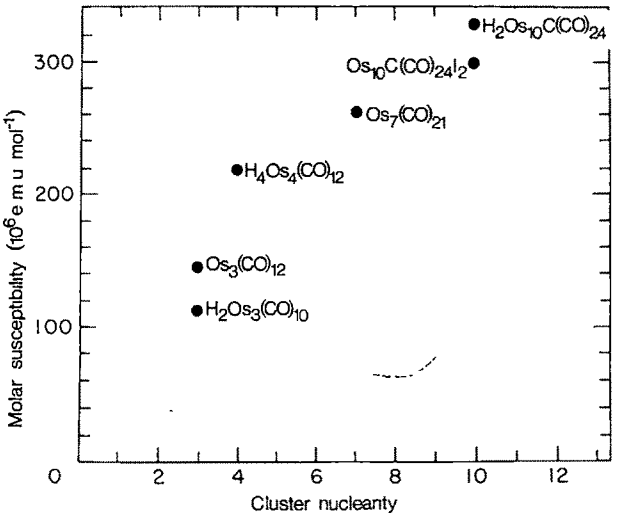


Fig. 2 Magnetism in Os cluster carbonyls. The variation of the high-temperature (298 K) excess molecular susceptibility, χ_{ems}, as a function of cluster nuclearity.

orbital study has been made[33] on $Ru_3(CO)_{12}$, isostructural with $Os_3(CO)_{12}$, to aid interpretation of its ultraviolet/visible electronic spectrum[34]. The first excited state in this D_{3h} molecule lies ~3 eV (24,000 cm^{-1}) above the ground state. Although optical transitions to this particular state are forbidden, it is of the correct symmetry to give a non-zero value for $|\langle n|\mu_z|0\rangle|^2$. This leads to a value for the TIP susceptibility of $\sim 180 \times 10^{-6}$ e.m.u. mol^{-1}, if we estimate $|\langle n|\mu_z|0\rangle|^2$ from symmetry arguments[35], which is indeed consistent with our observed value for $Os_3(CO)_{12}$. The next three excited states correspond to allowed optical transitions, but are magnetically forbidden, so that the sum over excited states in the calculation of the excess molecular paramagnetism is dominated by the HOMO–LUMO interaction, in this case.

It is important to note that these cluster molecules are tightly bound and contain electronic energy levels with values of $(E_n - E_0)$ greatly different from those of the individual interacting metal centres[36]. If the cluster units were loosely bound, as in the case of van der Waals-type clusters of noble gas atoms[19,37], their magnetic properties would simply be those of an assembly of mononuclear metal carbonyl fragments. This is clearly not the case for the title systems.

The ultraviolet/visible spectra of larger clusters show a trend towards absorption at lower frequencies. Chini[32] has noted that this trend can be taken to reflect the separation of frontier orbitals, which clearly correlates with the colour sequences found in the Os clusters and carbonylmetallates. If excited states of suitable symmetry are present, this would give rise to an increased TIP susceptibility as the cluster size increases (Fig. 2). Unfortunately, a more detailed discussion would be premature, as our knowledge of the excited electronic states in cluster molecules is extremely limited[20]. Very few data are available for the electronic absorption spectra of high-nuclearity clusters. Satisfactory quantitative theoretical calculations on large many-electron clusters of metal atoms with large spin-orbit coupling constants cannot at present be performed. Even the relatively simple case of the dichromium molecule, Cr_2, has given rise to considerable disagreement among the results from different methods of calculation[38]. Extended Hückel molecular-orbital calculations using Rh atom parameters have been partially successful, but an incorrect number of cluster valence molecular orbitals was obtained[39] for (ligand-free) 10-atom clusters having the geometry of $H_2Os_{10}C(CO)_{24}$.

If one traces the evolution of the Van Vleck paramagnetic contribution from the situation when $(E_n - E_0) \gg kT$, to $(E_n - E_0) \ll kT$, then the second-order Zeeman effect transforms smoothly from temperature-independent paramagnetism, to a Curie-type paramagnetism[7,29]. For the situation in which the characteristic energy gap is sufficiently small to be comparable with kT, namely, $(E_n - E_0) \leqslant kT$, the resulting contribution to the magnetization gives a susceptibility contribution proportional to $<n|\mu_z|0\rangle|^2/kT$. This has the usual Curie form, although here the mechanism of magnetization is by the admixture of virtual excited states into the ground state, whereas for 'free' (Curie) spins, the mechanism is that of redistribution among the spin states[29]. Note that, in this case, the energy parameter $(E_n - E_0)$ does not enter into the paramagnetic contribution. In the intermediate regime, Figgis[27] has noted that the observed magnetic susceptibility contains contributions from both types of effect.

Table 1 Magnetic susceptibility data for Os clusters (298 K)*

Cluster	χ_m(obs) (10^6 e.m.u. mol^{-1})	χ_{ems} (10^6 e.m.u. mol^{-1})
$Os_3(CO)_{12}$	−170 ± 10	+146 ± 10
$H_2Os_3(CO)_{10}$	−188 ± 10	+114 ± 10
$H_4Os_4(CO)_{12}$	−200 ± 10	+219 ± 10
$Os_7(CO)_{21}$	−405 ± 20	+263 ± 20
$H_2Os_{10}C(CO)_{24}$	−580 ± 30†	+326 ± 30
$Os_{10}C(CO)_{24}I_2$	−700 ± 40	+304 ± 40

* All values are corrected for the susceptibility of the Spectrosil container. The experimental data are temperature-independent down to 1.5 K; except for $H_2Os_{10}C(CO)_{24}$†, which is temperature-dependent below ~150 K.

We have reported[40] that the decanuclear cluster $H_2Os_{10}C(CO)_{24}$ behaves in this fashion, revealing a strong temperature-dependent paramagnetism at low temperatures. In addition, preliminary magnetic measurements on several high-purity anionic decanuclear Os clusters also show a Curie-type paramagnetism in the magnetic susceptibility. In terms of the Van Vleck theory of magnetism, this strong paramagnetic susceptibility is nominally symptomatic of a diamagnetic state in which the energy gap separating the ground state and excited state for mixing is comparable with kT. However, we caution against accepting this statement as a rigorous characterization of $(E_n - E_0)$ from the susceptibility data, as the thermodynamic and magnetic properties depend on the overall pattern of energy levels[41–43]. In particular, it is not clear whether the assumption of Boltzmann-type statistics is indeed valid for a metal cluster of this size, that is, whether Fermi–Dirac statistics would not be more appropriate in this particulate metal regime.

An alternative description, much discussed in solid-state physics[1,11,12], approaches the problem from the bulk metallic regime and considers the consequences of the fragmentation of a metallic sample into a 'particulate' metal. Briefly, the finite extension of the electron wavefunction within a particulate metal causes a changeover of the electron levels from quasi-continuous to discrete. The results of theoretical calculations of the magnetic properties of particulate metals depend on the assumptions made concerning the overall distribution of energy levels, for example, whether they are equally spaced, or in a Poisson distribution, and whether or not degeneracy of levels is allowed[41,42]. The unitary ensemble calculation, most appropriate for metals (such as Os) with high spin-orbit coupling, is the least studied, but a transition from the temperature-independent Pauli paramagnetism of bulk metals to a Curie-type behaviour in the magnetic susceptibility has been predicted for both odd- and even-electron metals[12,41,42]. Experimental observations of enhanced low-temperature susceptibility have been made for particles of metals as diverse as lithium[44] (odd electron), magnesium[45] and platinum[42] (even electron).

In terms of its magnetic properties, the decanuclear cluster $H_2Os_{10}C(CO)_{24}$ may be representative of a small metallic particle in the so-called quantum-size effect regime.

We note that the structure of $Os_{10}C(CO)_{24}I_2$ is considerably more 'open' than the hydrido-cluster $H_2Os_{10}C(CO)_{24}$, because of the bonding contribution of the iodine atoms[46]. It may be more correct in this context to regard the iodo-cluster as an octanuclear cluster with two extra peripheral (edge bridging)

Os atoms. In $Os_{10}C(CO)_{24}I_2$, only temperature-independent paramagnetism is observed (Fig. 2).

The low-temperature paramagnetism of $H_2Os_{10}C(CO)_{24}$ has been further investigated by electron paramagnetic resonance (EPR). Spectra of polycrystalline $H_2Os_{10}C(CO)_{24}$ showed a broad almost-symmetrical lorentzian resonance centred at $g = 2.27 \pm 0.02$. As the temperature was raised, the peak-to-peak linewidth, ΔH_{pp}, increased from ~50 G at 18 K to ~80 G at 77 K.

EPR signals with characteristic dysonian (asymmetrical) lineshapes are given by conduction electrons in bulk metals[48]. The mechanism of electron spin relaxation in metals has been discussed in detail by Elliott[49,50], who showed that spin relaxation occurs through the combined agencies of electron–phonon scattering and spin–orbit coupling. For heavy bulk metals in which spin–orbit interactions are large, for example, Os with a spin–orbit coupling constant of ~1,000 cm^{-1}, electron spin relaxation times are expected to be extremely short and EPR resonances are anticipated to be broadened beyond detection at ambient temperatures[51]. Using values of the resistivity relaxation rate (τ_R^{-1}), derived from measurements on bulk Os metal, and taking $g = 2.27$, gives an electron spin lattice relaxation rate of $2 \times 10^{14}\ s^{-1}$; the corresponding EPR linewidth would be $\sim 1 \times 10^7$ G, clearly undetectable. But a relaxation rate of $\sim 1 \times 10^9\ s^{-1}$ is indicated from the EPR of $H_2Os_{10}C(CO)_{24}$.

This large reduction in electron spin relaxation rate from the bulk Os value is also consistent with a description of $H_2Os_{10}C(CO)_{24}$ as a particulate metal. It is well established that electron spin relaxation processes are inhibited or suppressed in this quantum-size effect regime[12,52]. Briefly, in a particulate metal the electronic energy level spacing may become comparable with the magnetic Zeeman energy. Quenching of the electron spin relaxation processes in small metallic particles arises because electron (spin) transitions between these discrete energy levels[52] are restricted as opposed to the situation in a bulk metallic sample[49]. Therefore, this quantum-size effect considerably narrows the EPR absorption and it may become detectable. Moreover, if the particle diameter is smaller than the characteristic microwave skin depth, the lineshape changes from dysonian to lorentzian[48,50]. EPR signals of lorentzian shape have indeed been observed for small particles of metals with both odd (for example, alkalis[50], silver[53–55]) and even (for example, magnesium[44] and platinum[56]) numbers of valence electrons. Similarly, small metallic particles studied[57] by EPR in alkali halide (host) crystals include the (even electron) 2nd row transition metals Pd and Cd. There have been no reports of Os colloids isolated in these host materials.

Conclusions

This study of the magnetic properties of a series of Os clusters demonstrates experimentally the manner in which the changeover from molecular to 'particulate-metal' behaviour may develop as a cluster aggregate forms. The temperature-independent Van Vleck paramagnetism increases sequentially with cluster size as the molecular electronic states begin to develop into the band or continuum regime and ultimately terminates in the characteristic Pauli paramagnetism in the bulk metallic state. Moreover, electronic states within the particulate-metal regime, in which quantum-size effects may be operative, can exhibit enhanced paramagnetism compared with both their localized- and itinerant-electron counterparts. Similar observations have been made on particulate samples of several metals. There are other theoretical predictions and experimental observations of enhanced paramagnetism in this 'narrow-band' regime separating the metallic and insulating states[58,59].

We have demonstrated the considerable sensitivity of magnetic measurements in detecting the onset of certain aspects of what may be termed 'metallic' behaviour. This has an important bearing on the question[6], 'how many atoms are needed in an aggregate or cluster to mimic effectively the bulk properties of a metal'? The answer must obviously depend on which particular property of the metal one wishes to describe. Transport properties (for example, conductivity, Hall effect) require, as a prerequisite for detection of metallic character, the complete ionization of an electron from its parent site, which typically involves energy changes in the range 10^{-1}–10 eV. As far as the magnetic properties are concerned, modifications to the intra- and inter-unit exchange energy in the metal cluster as the nuclearity increases need only be of the order of magnitude of the magnetic Zeeman or hyperfine energies (both typically in the range 10^{-4} eV) for noticeable changes to be observed. These types of experimental considerations are fundamental to any consideration of 'metallic' characteristics. Several authors[1,7–10,60] have suggested a criterion for bulk properties in clusters based on the width of the electron d band, as determined experimentally, for example, by photoelectron spectroscopy. Such a criterion obviously involves large energy terms in the range 1–10 eV. Similarly, quantum mechanical calculations aimed at reproducing bulk-metal d-band widths in clusters seem certain to require several hundreds, or even thousands, of atoms before metallic status would be conferred on the aggregate[7–10]. These approaches may overlook and obscure many interesting magnetic and optical properties of clusters in the particulate-metal regime.

We have suggested that transition metal carbonyl cluster compounds represent ideal model systems for monitoring the genesis of a particulate metal from individual molecular units. Transition metal cluster compounds of slightly higher nuclearity than those examined here may show very interesting electronic properties, possibly arising from a polarization catastrophe[6] within the cluster units themselves.

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1. Borel, J-P & Buttet, J (eds) *Small Particles and Inorganic Clusters, Surf. Sci.* **106**, (1981)
2. Davis, S C & Klabunde, K J *Chem Rev* **82**, 153–208 (1982)
3 Maugh, T H *Science* **219**, 474–477 (1983)
4 Bourdon, J (ed) *Growth and Properties of Metal Clusters, Proc. 32nd int Meet Soc Chim. Phys* Villeurbanne (Elsevier, Amsterdam, 1980)
5. Muetterties, E L, Rhodin, T N, Band, E, Bruker, C F & Pretzer, W R *Chem Rev* **79**, 91–137 (1979).
6 Edwards, P P & Sienko, M J *Int Rev phys Chem.* **3**, 83–137 (1983).
7 Baetzold, R C *Inorg Chem* **20**, 118–123 (1981)
8 Bachmann, C, Demuynck, J & Veillard, A *Faraday Symp. chem. Soc.* **14**, 170–179 (1980)
9 Demuynck, J, Rohmer, M-M, Strich, A & Veillard, A *J chem. Phys* **75**, 3443–3453 (1981).
10. Yang, C Y, Johnson, K H, Salahub, D R, Kaspar, J & Messmer, R P *Phys Rev* **B24**, 5673–5692 (1981)
11 Fröhlich, H *Physica* **4**, 406–412 (1937)
12 Kubo, R *J. phys Soc Japan* **17**, 975–986 (1962), *J Phys, Fr* **38**, Suppl 7, C2-69 (1977)
13 Abeles, B, Sheng, Ping, Coutts, M D & Arie, Y *Adv Phys* **24**, 407–461 (1975)
14 Ozin, G A *Faraday Symp chem. Soc.* **14**, 7–64 (1980)
15 Howard, J, Sutcliffe, R, Tse, J S & Mile, B *Chem. Phys Lett* **94**, 561–564 (1983)
16 Thompson, G A, Tischler, F & Lindsay, D M *J chem Phys* **78**, 5946–5953 (1983)
17 Johnson, B F G (ed) *Transition Metal Clusters* (Wiley, Chichester, 1980)
18. Johnson, B. F G & Lewis, J *Adv inorg Chem. Radiochem* **24**, 225–355 (1981)
19 Hoare, M R & Pal, P *Nature Phys Sci* **236**, 35–37 (1972), *Adv. Phys* **24**, 645–678 (1975)
20 Woolley, R G *Nouv J Chim.* **5**, 441–446 (1981), in *Transition Metal Clusters* (ed Johnson, B F G) 607–659 (Wiley, Chichester, 1980)
21 Yvon, K *Curr Topics Mater Sci* **3**, 53–129 (1979)
22 Johnson, D C thesis, Cornell Univ (1983)
23 Konig, E in *Landolt-Bornstein, New Ser, Group II Atomic and Molecular Physics* Vol 2 (ed Hellwege, K.-H) 1 (Springer, Berlin, 1966).
24 Freed, S & Kasper, C. *J Am chem Soc.* **52**, 4671–4679 (1930)
25 Carlin, R H & van Duyneveldt, A J. *Magnetic Properties of Transition Metal Compounds* (Springer, New York, 1977)
26 Van Vleck, J H *Electric and Magnetic Susceptibilities* (Oxford University Press, 1932).
27. Figgis, B N *Introduction to Ligand Fields*, 252–256 (Interscience, New York, 1966)
28 Freeman, R, Murray, G. R & Richards, R E *Proc. R. Soc.* **A242**, 455–466 (1957)
29. Kittel, C *Introduction to Solid State Physics* 2nd edn 578–579 (Wiley, New York, 1963).
30 Wade, K in *Transition Metal Clusters* (ed Johnson, B F G) Ch 3 (Wiley, Chichester, 1980)
31 Mingos, D. M P. *Nature phys Sci* **236**, 99–102 (1972)
32 Chini, P *Pure appl. Chem.* **23**, 489–503 (1970)
33 Delley, B, Manning, M C, Ellis, D E, Berkowitz, J & Trogler, W C *Inorg Chem* **21**, 2247–2253 (1982)
34 Tyler, D R, Levenson, R. A & Gray, H B *J Am. chem Soc.* **100**, 7888–7893 (1978)
35 Ballhausen, C. J *Introduction to Ligand Field Theory*, 147 (McGraw-Hill, New York, 1962)
36 Hoffmann, R, Schilling, B E R, Bau, R., Kausz, H & Mingos, D M P *J Am chem. Soc.* **100**, 6088–6093 (1978)
37. Jortner, J *Ber Bunsenges. Phys Chem.* **88**, 188–201 (1984)
38 Michalopoulos, D. L, Geusic, M E, Hansen, S G, Powers, D. E & Smalley, R E *J phys Chem.* **86**, 3914–3916 (1982)
39. Ciani, G & Sironi, A *J organomet Chem* **197**, 233–248 (1980).

40 Benfield, R E, Edwards, P P & Stacy, A M *JCS chem Commun*, 525-526 (1982)
41 Denton, R, Muhlschlegel, B & Scalapino, D J *Phys Rev Lett* **26**, 707-711 (1971). *Phys Rev* B7, 3589-3607 (1973)
42 Marzke, R F *Catal Rev Sci Engng* **19**, 43-65 (1979)
43 Knight, W D *J Vacuum Sci Technol* **10**, 705-712 (1973)
44 Borel, J-P & Millet, J-L *J Phys, Fr* **38**, 115-119 (1977)
45 Millet, J-L & Borel, J-P *Surface Sci* **106**, 403-407 (1981)
46 Lewis, J & Johnson, B F G *Pure appl Chem* **54**, 97-112 (1982)
47 Dyson, F J *Phys Rev* **98**, 349-359 (1955)
48 Feher, G & Kip, A F *Phys Rev* **98**, 337-348 (1955)
49 Elliott, R J *Phys Rev* **96**, 266-279 (1954)
50 Edmonds, R N, Guy, S C, Edwards, P P & Johnson, D C *J phys Chem* (in the press), Edmonds, R N & Edwards, P P *Proc R Soc* (in the press)
51 Monod, P & Beuneu, F *Phys Rev* B**18**, 2422-2425 (1978), **19**, 911-916 (1979)
52 Kawabata, A *J phys Soc. Japan* **29**, 902-911 (1970)
53 Millet, J.-L. & Borel, J-P *Solid St. Commun* **43**, 217-220 (1982)
54 Hermerschmidt, D & Haul, R *Ber Bunsenges phys Chem* **84**, 902-907 (1980)
55 Ozin, G A *J Am chem Soc.* **102**, 3301-3303 (1980)
56 Gordon, D A, Marzke, R F & Glaunsinger, W S *J Phys, Fr* **38**, C2-87-91 (1977)
57 Hughes, A E & Jain, S C *Adv Phys* **28**, 717-828 (1979)
58 Mott, N F *Metal-Insulator Transitions* (Taylor & Francis, London, 1974)
59 Holcomb, D F in *The Metal-Non-Metal Transition in Disordered Systems* (eds Fredman, L P & Tunstall, D P) 251-284 (19th Scottish Universities Summer School in Physics, Edinburgh, 1978)
60 Hamilton, J F, Apai, G, Lee, S T & Mason, M G in *Growth and Properties of Metal Clusters* (ed Bourdon, J) 387-391 (Elsevier, Amsterdam, 1980).

Hydrogen bonding and biological specificity analysed by protein engineering

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The role of complementary hydrogen bonding as a determinant of biological specificity has been examined by protein engineering of the tyrosyl-tRNA synthetase. Deletion of a side chain between enzyme and substrate to leave an unpaired, uncharged hydrogen-bond donor or acceptor weakens binding energy by only 0.5–1.5 kcal mol^{-1}. But the presence of an unpaired and charged donor or acceptor weakens binding by a further ~3 kcal mol^{-1}.

THE hydrogen bond is a ubiquitous feature of biological interactions: it is essential in determining the structure of proteins and nucleic acids; it is a major determinant of specificity in enzyme catalysis and in biological information transfer; and it can influence directly the rate of enzymatic reactions by stabilizing the ionic charges formed in the transition state. Hydrogen bonding in macromolecules and their complexes in aqueous solution is a complex phenomenon because water competes for the hydrogen-bonding sites[1,2]. The calculation of the overall energetics is consequently difficult and there is little knowledge of the energies involved[3]. Here we apply an experimental approach to the problem, using site-directed mutagenesis to produce mutant enzymes that differ in their abilities to form hydrogen bonds with their substrates. The interaction energies can be calculated from kinetic data on the modified enzymes.

Our experimental system is the tyrosyl-transfer RNA synthetase from *Bacillus stearothermophilus*. This enzyme catalyses the aminoacylation of $tRNA^{Tyr}$ in a two-step reaction; activation of the amino acid to form the enzyme-bound tyrosyl adenylate complex followed by transfer to $tRNA^{Tyr}$ (ref. 4).

$$E + Tyr + ATP = E.Tyr\text{-}AMP + PP_i \quad (1)$$

$$E\ Tyr\text{-}AMP + tRNA = Tyr\text{-}tRNA + AMP \quad (2)$$

The particular suitability of this enzyme (E) for obtaining accurate kinetic data and its other favourable characteristics for systematic site-directed mutagenesis have been described in detail elsewhere[5]. The three-dimensional structure of the enzyme and its bound aminoacyl adenylate are known from X-ray crystallography[6–9]. Eleven possible hydrogen bonds have been identified that may be formed between the enzyme and substrate, eight of which are from amino-acid side chains that may be mutated (Fig 1). We are systematically mutating these residues to measure the energetics of their interactions and to test whether hydrogen bonds are actually involved[10]. In all cases, the mutations lead to a smaller side chain that either lacks or has a modified hydrogen-bond donor or acceptor. Our initial data are consistent with hydrogen bonding being an exchange reaction whereby the hydrogen-bond donors and acceptors on the free enzyme and free substrate break their bonds with water on forming the bonds in the enzyme-substrate complex[5,11,12]. Here we analyse the effects of mutation of several different types of hydrogen bonds, including bonds from residues involved in determining the high specificity of the enzyme for tyrosine rather than phenylalanine (Tyr 34), bonds for simple binding of the substrate (Cys 35, His 48, Thr 51, Tyr 169) and a bond closer to the seat of reaction (Gln 195). These bonds may be classified further according to charge; most are between uncharged polar residues, but some involve a charged group on either the enzyme or substrate.

Experimental observations

Our experimental results for the activation of tyrosine are listed in Table 1. The contributions of the side chains to binding were calculated from the values of k_{cat}/K_M as described previously (ref. 5, Table 2). Note that the data refer to the binding of the substrate in the transition state. Such data give, in general, the most reliable measurements of incremental binding energies as the effects of strain, nonproductive binding and induced fit do not affect k_{cat}/K_M (ref. 13). The data for the activation of phenylalanine by the wild-type enzyme and a mutant in the specificity pocket are listed in Table 3. The contributions of side chains to binding energy, discussed in more detail below, may be summarized and classified thus: (1) deletion of a side chain on the enzyme that forms a good hydrogen bond with an uncharged group on the substrate weakens binding energy by only 0.5–1.5 kcal mol^{-1}; (2) deletion of an uncharged side chain on the enzyme that forms a hydrogen bond with a charged group on the substrate weakens binding by ~3.5–4.5 kcal mol^{-1}—only two relevant experiments were performed for this category and so the range could be much wider, but the crucial point is that these energies are considerably higher than in (1); (3) deletion of a group that forms a too-long hydrogen bond actually improves binding.

Effects of removal of side chains

Our measurements on the mutated enzymes give the overall contributions of the side chains to binding. The hydrogen bond donors/acceptors that are removed on mutagenesis are the only portions of the side chains in direct contact with the substrate. But the mutation of an amino acid in a protein from a larger side chain to a smaller may have further structural consequences superimposed on the energetics of the hydrogen bonding. Any fine structure analysis must consider these possibilities and

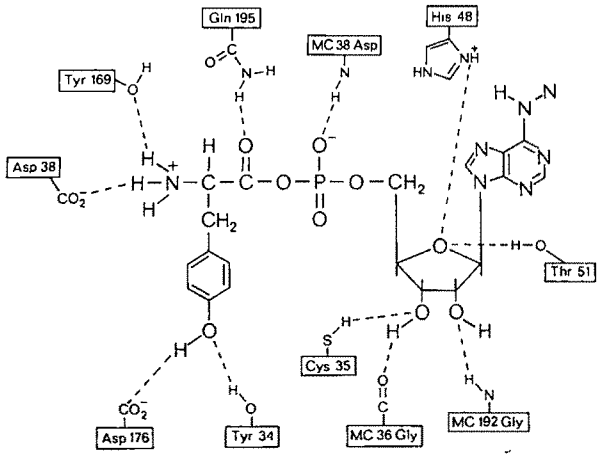


Fig. 1 Hydrogen bonds between the tyrosyl-RNA synthetase and tyrosyl adenylate. MC, main chain.

attempt to eliminate artefacts. The obvious experimental procedure is to crystallize the mutants and use protein crystallography to detect structural changes. Such a technique will detect changes of ~0.1 Å or more but will miss a series of smaller changes. (The effects of small relaxations of protein structure on binding are not known.)

Because kinetics measurements are exceptionally sensitive and can detect changes of a fraction of a kilocalorie, we use kinetic methods to increase the reliability of analysis. For example, application of the 'double-mutant' procedure of Carter *et al.*[14] has shown that mutation of residues 35 and 48 does not cause structural changes propagated through the protein[14]. Experiments in which we have mutated side chains by the removal of methyl groups buried in the protein, however, show that these small changes can weaken binding by up to 1 kcal mol^{-1} (A.J.W., A.R.F., P.C. and G.W., unpublished data). Because of this, we have analysed many different mutants to observe trends. The combination of the extensive and consistent kinetic data (see below) and modelling by molecular graphics suggests that the results so far reflect the direct effects of hydrogen bonding itself and are not dominated by structural change.

Hydrogen-bond strengths *in vacuo*

The stabilization energies of hydrogen bonds *in vacuo*, between compounds X-H and B-Y in equation (3), have been calculated[15]:

$$\text{X-H}\cdots\text{B-Y} = \text{X-H} + \text{B-Y} \qquad (3)$$

(where −H is a hydrogen-bond donor and −B is an acceptor). Representative values for the energies of stabilization for different donors and acceptors are: water/water, −6.4 kcal mol^{-1}; water/CH_3SH, −3.1 kcal mol^{-1} for −SH as the acceptor, −3.2 kcal mol^{-1} for −SH as the donor; imidazolium/water, −14 kcal mol^{-1}; acetate/water, −19.8 kcal mol^{-1}. These values are much greater than those in Table 2 for the overall energies of bonding in aqueous solution.

General model for H bonding in water

Our data fit the formulation of Jencks[16] and Hine[17]:

$$\text{E-H}\cdots\text{OH}_2 + \text{HOH}\cdots\text{B-S} = \text{E-H}\cdots\text{B-S} + \text{HOH}\cdots\text{OH}_2 \qquad (4)$$

For convenience, we assume that the enzyme has a hydrogen-bond donor, −H, and the substrate (S) an acceptor, −B, that pair in the enzyme-substrate complex. In the free enzyme, −H and −B are bound to water molecules. The number and types of hydrogen bonds are conserved in the reaction, which is thus essentially isoenthalpic (within the limits of Hine's[17] equation). For example, as discussed by Wilkinson *et al.*[5], a −SH group is just as effective as a −OH group as a hydrogen-bond donor/acceptor, despite the different absolute strengths of −SH···O and −OH···O bonds in equation (3); if, in equation (4), −H is part of a −SH group, then there is a −SH···O bond

Table 1 Activation of tyrosine by tyrosyl-tRNA synthetase and its mutants

Enzyme	k_{cat} (s^{-1})	K_M (ATP) (mM)	K_M (Tyr) (μM)	k_{cat}/K_M (ATP) ($s^{-1}\,M^{-1}$)	k_{cat}/K_M (Tyr) ($s^{-1}\,M^{-1}$)
Wild type	8.35	1.08	2 23	7,730	3.74×10^6
Tyr→Phe 34	6.86	1.2	4.4	5,720	1.56×10^6
Cys→Gly 35	2.95	2.6	2.7	1,130	$1\,09\times10^6$
Cys→Ser 35	2.52	2.4	2.6	1,050	9.65×10^5
His→Asn 48	7.90	1.4	3.8	5,640	2.08×10^6
His→Gly 48	2.00	1.3	3.2	1,540	6.25×10^5
Thr→Ala 51	8.75	0.54	2.0	16,200	4.36×10^6
Gln→Gly 195	0.19	2.5*	100	76*	1.90×10^3
Tyr→Phe 169†	6 05	1.25‡	1,030	4,840‡	5.88×10^3
Δ(321-419)†	7.50	1.08	2.4	6,940	3.12×10^6

Mutant enzymes were prepared, assayed and active-site titrated as described previously[5,14]. Activation was measured by pyrophosphate exchange at 25 °C, *p*H 7.78 in the presence of 144 mM Tris-Cl, 10 mM $MgCl_2$ and 2 mM PP_i. Kinetic constants for variation of ATP were determined in the presence of 0.05 mM tyrosine (except where indicated otherwise) and for tyrosine in the presence of 2 mM ATP. Values of k_{cat} are extrapolated to infinite concentration of tyrosine and ATP.

* 0.3 mM tyrosine.

† Experiments on truncated enzyme with residues 321–419 deleted (the tRNA binding domain)[22].

‡ 1.7 mM tyrosine.

on the left-hand side and a −SH···O bond on the right in the enzyme-substrate complex. The contribution of hydrogen bonds to enzyme-substrate binding energy arises from entropy; water hydrogen bonded to the enzyme or substrate has lower entropy than bulk water and the release of hydrogen-bonded water helps drive enzyme-substrate binding[13,16]. Thus, there is a crucial distinction between the absolute energy of a hydrogen bond as in equation (3) and the overall energetics of hydrogen bonding in solution. It is the overall energetics that are important in the binding of enzymes and substrates, and it is the overall energetics that we measure from site-directed mutagenesis.

The removal of one of the hydrogen-bonding groups from equation (4), for example a side chain of the enzyme as in equation (5),

$$\text{E}\,\text{OH}_2 + \text{HOH}\cdots\text{B-S} = [\text{E B-S}] + \text{HOH} + \text{OH}_{2(\text{bulk})} \qquad (5)$$

does not necessarily lead to the loss of the absolute binding energy (enthalpy) of a hydrogen bond as defined by equation (3). Deletion of a hydrophilic side chain means that a water molecule must be located next to a hydrophobic region of the enzyme in the space vacated. This water molecule is at a high energy because it is at an interface and not in bulk water where it can fulfil readily all its hydrogen bonding. There is no hydrogen bond from E to H_2O in the free enzyme and so this partly (or even fully) compensates for the lack of the hydrogen bond from E to −B in the enzyme-substrate complex. When the substrate binds, it displaces the high-energy water molecule (which returns to the bulk solvent), and the hydrophilic hydrogen-bond acceptor of the substrate occupies the 'hydrophobic' site. The overall energetics of the reaction in equation (5) depend on the precise interactions made by the mutant enzyme with the water molecule and the group −B on the substrate. If, for example, the enzyme-bound water molecule in equation (5) is in the same orientation as that in equation (4), then equation (5) may be written as

$$\text{E}\,\text{OH}_2 + \text{HOH}\cdots\text{B-S} = [\text{E B-S}] + \text{HOH}\cdots\text{OH}_2 \qquad (6)$$

There is one hydrogen bond on the right and a similar one on the left of equation (6). This reaction is approximately isoenthalpic when −B is −OH and, to a first approximation, the energetics of equation (6) are the same as those of equation (4). Therefore, deletion of the hydrogen bond in the enzyme-substrate complex does not lead to the overall loss of the absolute strength of a hydrogen bond and may, in certain circumstances, cause no loss of binding energy. The energetics clearly depend on the precise nature of the interactions in each particular example and so will

Table 2 Comparative binding energies of wild-type and mutant enzymes with substrates

Comparison (Residue and position)		Substrate	ΔG (kcal mol^{-1})
Phe 34	Tyr 34*	Tyr	0.52
Gly 35	Cys 35*	ATP	1.14
Ala 51	Cys 51	ATP	0.47
Gly 48	Asn 48	ATP	0.77
Gly 48	His 48*	ATP	0.96
Ser 35	Cys 35*	ATP	1.18
Phe 169†	Tyr 169*†	Tyr	3.72
Gly 195	Gln 195*	Tyr	4.49
Gly 35	Ser 35	ATP	−0.04
Ala 51	Thr 51*	ATP	−0.44

The first two columns show the residues compared. The apparent contributions (ΔG) of the side chains of different amino acids to the binding energy of the enzyme–transition state complexes were calculated by comparing the ratios of k_{cat}/K_M for activation by wild-type and mutant enzymes as described previously[5], using the equation $\Delta G = -RT \ln\{(k_{cat}/K_M)_{mut}/(k_{cat}/K_M)_{wt}\}$ (subscripts mut and wt refer to mutant and wild-type (or reference) enzymes respectively).

* Wild-type; † truncated enzyme[22].

Table 3 Activation of tyrosine and phenylalanine by tyrosyl-tRNA synthetases

Enzyme	Activation of Tyr			Activation of Phe	$\frac{(k_{cat}/K_M)_{Tyr}}{(k_{cat}/K_M)_{Phe}}$
	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($s^{-1}M^{-1}$)	k_{cat}/K_M ($s^{-1}M^{-1}$)	(Relative specificity)
Wild type	5.4	2.2	2.5×10^6	17	1.5×10^5
Tyr→Phe 34	4.4	4.4	1.0×10^6	100	1.0×10^4

Impurities of tyrosine in the phenylalanine were removed by scavenging with tetranitromethane[23]. Conditions as described in Table 1 but data are not extrapolated to infinite concentration of ATP but given for 2 mM ATP. For activation of phenylalanine, the Phe concentration was varied between 5 and 30 mM, well below the K_M value. The specificity of wild-type enzyme is close to that measured previously under different conditions[23].

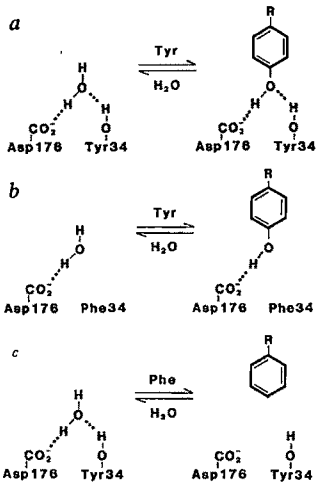


Fig. 2 Illustration of the hydrogen-bond inventory on tyrosine and phenylalanine binding to the tyrosyl-tRNA synthetase. *a*, Binding of wild-type enzyme and tyrosine involves the formation of two hydrogen bonds between its hydroxyl and Asp 176 and Tyr 34, but two equivalent hydrogen bonds of Asp 176 and Tyr 34 with water are broken. *b*, Binding of tyrosine to the Tyr→Phe 34 mutant involves just the formation of one hydrogen bond with the enzyme, but only one hydrogen bond is broken on displacing the bound water molecule. *c*, The binding of phenylalanine to the enzyme with the concomitant displacement of water would lose a strong hydrogen bond between water and Asp 176 and lead to a partially desolvated charge between the enzyme and substrate. Also, Tyr 34 would be unsolvated. Further, when tyrosine is bound, there are van der Waals' interactions between its phenolic hydroxyl and the enzyme, which are not present when phenylalanine is bound. It is probable that phenylalanine binds to the enzyme with the water molecule remaining bound between Asp 176 and Tyr 34. Note that in *a* and *b* the water molecules released from the enzyme on the binding of tyrosine can be considered to take the place of the tyrosine hydroxyl in solution so that there is no net change in the number of hydrogen bonds in solution.

vary from case to case. Before this study, the magnitudes of the energetic changes were unknown. Now we discuss these and show how particular examples may be rationalized and categorized.

Case A. Deletion of a side chain (−H) that interacts with an uncharged group on the substrate. Equation (5) or (6) describes this. The low contribution to specificity of a hydrogen bond between two uncharged residues is exemplified by the mutation Tyr→Phe 34 (Figs 1, 2). This is a very conservative mutation whereby a small group is deleted whose interaction is important in the recognition of tyrosine compared with phenylalanine. Yet, K_M for tyrosine increases by a factor of only two (Table 1) and the specificity for tyrosine decreases by a factor of only 15 (Table 3). The apparent bond energy of 0.5 kcal mol^{-1} is one of the lowest yet found, perhaps because the tyrosine −OH is a poorer hydrogen-bond acceptor than an aliphatic or water −OH and so the bond from Tyr 34 to the tyrosyl −OH of the substrate is relatively weak. The thiol-containing side chain from Cys 35, which donates to the 3′-OH of the ribose of ATP, contributes 1.1 kcal mol^{-1}. The tyrosyl-tRNA synthetase from *Bacillus caldotenax* differs from the enzyme from *B. stearothermophilus* by only four amino-acid replacements (M.D. Jones, unpublished data). One of these is an asparagine at position 48. The side chain of Asn 48 contributes 0.77 kcal mol^{-1} and that of His 48 contributes slightly more binding energy at 0.96 kcal mol^{-1}.

Note that equation (5) is relatively insensitive to the nature of the side chain deleted (when −B is uncharged). For example, consider the deletion of a charged histidine side chain (−H is imidazolium) as in the mutation His→Gly 48. Then, relative to wild-type enzyme, a strong interaction between imidazolium and the substrate is lost in the mutant enzyme–substrate complex, but this is compensated for by a loss of a strong interaction between imidazolium and water in the free mutant enzyme.

We have analysed several mutants as well as those listed in Table 2. In all cases, the deletion of a side chain that interacts with an uncharged group on the substrate loses between 0.5 and 1.5 kcal mol^{-1} of binding energy.

Case B. Deletion of a side chain that interacts with a charged group on the substrate. This is exemplified by the mutation Tyr→Phe 169 (Fig. 1) whereby the hydrogen bond to the ammonium ion is lost in the enzyme–substrate complex. Equation (5) still describes the situation, but there is now the loss of a strong hydrogen bond between the charged group on the substrate and water when forming the enzyme–substrate complex and 3.7 kcal mol^{-1} of binding energy are lost on Tyr→Phe 169. Gln 195 binds to a carboxylate oxygen of tyrosine in the E.Tyr complex and to the carbonyl oxygen of tyrosyl adenylate (Fig. 1; refs 6, 7, 18). On mutation of Gln→Gly 195 there is a loss of binding energy of 4.5 kcal mol^{-1} in the transition state where the oxygen atom is fractionally charged.

Case C. Deletion of a group on the substrate that interacts with a charged residue on the enzyme, described by the equation.

$$E\text{-}H^+\cdots OH_2 + HOH\,S = [E\text{-}H^+\,S] + HOH + OH_{2(bulk)} \quad (7)$$

This has similar consequences to Case B. A strong interaction in the enzyme–water complex is lost, an example being the 'modification' of tyrosine to phenylalanine. Phenylalanine binds more poorly to the enzyme by ~7 kcal mol^{-1} (Table 3). The phenylalanine either displaces the water molecule that is bound between Asp 176 and Tyr 34 in the enzyme (Fig. 2), leaving two unpaired hydrogen bonds, or, more probably, binds with a water molecule still bound between Asp 176 and Tyr 34. The water is crammed in a space which is too small, so that there are unfavourable interactions with the substrate.

A minimum estimate of the strength of the bond between Asp 176 and the tyrosine −OH can be made from the relative binding of tyrosine and phenylalanine to the mutant Tyr→Phe 34 (5.45 kcal mol^{-1} calculated from the data in Table 3). If phenylalanine displaces the bound water molecule as in Fig. 2, then there will be the loss of the hydrogen bond and also of the dispersion energy between the enzyme and the −OH group. The latter is worth ~1–2 kcal mol^{-1} (ref. 19) so the hydrogen bond

is worth 3.5–4.5 kcal mol^{-1}. If it is easier to bind phenylalanine without displacing the water, then the bond must be stronger than this.

Case D. Deletion of a side chain when there are geometrical constraints against hydrogen bonding in an enzyme–substrate complex. The above cases assume that there are no constraints on forming hydrogen bonds in the enzyme–substrate complex. When, as in equation (8), there is poor hydrogen bonding

$$\text{E-H}\cdots\text{OH}_2+\text{HOH}\cdots\text{B-S}=[\text{E-H B-S}]+\text{HOH}\cdots\text{OH}_2 \quad (8)$$

because the intermolecular distance between −H and −B is too great, there is just one good hydrogen bond on the right-hand side but two on the left of the equation. Deletion of −H may increase the affinity of the enzyme for the substrate because with the mutant enzyme there is one good hydrogen bond on the right but now only one on the left. Mutation of Thr→Ala 51 (ref. 12) is an example of this, as is Ser→Gly 35.

Case E. Deletion of a side chain on the enzyme or a group on the substrate that allows access of water between enzyme and substrate in their complex. It was suggested in Case C that phenylalanine binds to the tyrosyl-tRNA synthetase without displacing the bound water molecule but with unfavourable van der Waals' repulsion. On deletion of a large side chain from the enzyme, there could perhaps be adequate room for a water molecule to occupy comfortably the gap left by the deleted side chain, as in equation (9).

$$\text{E HOH}\cdots\text{OH}_2+\text{HOH}\cdots\text{B-S}=\text{E HOH}\cdots\text{B-S}+\text{HOH}\cdots\text{OH}_2 \quad (9)$$

A hydrogen-bond inventory shows that the number of hydrogen bonds may be formally conserved, as in Case A, equation (6). The energetics are expected to be similar to Case A for −B being an uncharged side chain. When −B is charged, as in Case B, the retention of solvent water by the charged group in the mutant enzyme–substrate complex will tend to attenuate the effects described in Case B.

Biological specificity

The classical view of biological specificity is that binding energy is provided by dispersion forces and the hydrophobic effect, whilst complementary hydrogen bonds and salt bridges give specificity[16,20]. This study shows that, irrespective of any small structural effects that occur on deletion of side chains, a side chain that forms a hydrogen bond between the enzyme and an uncharged group on a substrate provides only 0.5–1.5 kcal mol^{-1} of binding energy relative to the absence of the side chain. This means that an unpaired uncharged hydrogen bonder provides a factor of only 2.5–15 or so towards specificity. But the absence of a group on the enzyme or a substrate that should form a hydrogen bond with a charged group (as in Cases B and C) affects binding by ~4 kcal mol^{-1} and is worth a factor of 1,000 in specificity. Thus, specificity is caused to some extent by hydrogen bonding but is best mediated by charged residues.

In principle, the same reasoning may be applied to the contribution of hydrogen bonding in base pairing during DNA replication or transcription. There is, however, one important difference when analysing, for example, G·T and A·C mispairing. All the cases discussed here concern the removal of groups so that no unfavourable steric interference is set up between the enzyme and substrate. But, if in base mispairing, G·T takes up the same overall geometry as an A·T base pair and A·C the same as a G·C pair, unfavourable steric and electrostatic interactions will be set up between >NH groups. These effects will compound the loss of hydrogen bonds in the mispairs. In general, unfavourable steric interactions are an important element in specificity, as van der Waals' repulsion energies are such a strong function of interatomic distance. High specificity by steric repulsion is the basis of double-sieve sorting of amino acids by the aminoacyl-tRNA synthetases[21]. Thus, two important components of biological specificity are the avoidance of unsolvated charges and unfavourable steric interactions between enzyme and substrate.

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1 Kauzmann, W *Adv Protein Chem.* **14**, 1-63 (1959)
2 Klotz, I M & Franzen, J S *J Am chem Soc.* **84**, 3461-3466 (1962)
3 Cantor, C R & Schimmel, P R *Biophysical Chemistry* pt 1, 277 (Freeman, San Francisco, 1980)
4 Fersht, A R & Jakes, R. *Biochemistry* **14**, 3350-3356 (1975)
5 Wilkinson, A J, Fersht, A. R, Blow, D M & Winter, G. *Biochemistry* **22**, 3581-3586 (1983)
6. Monteilhet, C & Blow, D M *J molec. Biol* **22**, 407-417 (1978)
7 Rubin, J & Blow, D M *J molec. Biol* **145**, 489-500 (1981)
8 Bhat, T N, Blow, D M, Brick, P & Nyborg, J *J molec Biol* **158**, 699-709 (1982)
9 Blow, D M & Brick, P in *Biological Macromolecules and Assemblies* Vol 2 (eds Jurnak, F & McPherson, A) 442-469 (Wiley, New York, 1985)
10 Fersht, A R *et al Angew Chem* **23**, 467-473 (1984)
11 Winter, G, Fersht, A R, Wilkinson, A J, Zoller, M & Smith, M *Nature* **299**, 756-758 (1982)
12 Wilkinson, A J, Fersht, A R, Blow, D M, Carter, P & Winter, G *Nature* **307**, 187-188 (1984)
13 Fersht, A *Enzyme Structure and Mechanism* Ch 2 (Freeman, New York, 1985)
14. Carter, P J, Winter, G, Wilkinson, A J & Fersht, A R *Cell* **38**, 835-840 (1984)
15 Weiner, S J *et al. J Am. chem Soc.* **106**, 765-784 (1984)
16 Jencks, W. P *Catalysis in Chemistry and Enzymology* (McGraw-Hill, New York, 1969)
17 Hine, J *J Am. chem Soc.* **94**, 5766-5771 (1972).
18 Monteilhet, C, Blow, D M & Brick, P *J molec. Biol* **173**, 477-485 (1984)
19 Fersht, A R & Dingwall, C. *Biochemistry* **18**, 1245-1249 (1979)
20 Fersht, A. R *Trends biochem Sci* **9**, 145-147 (1984)
21 Fersht, A R & Dingwall, C *Biochemistry* **18**, 2627-2631 (1979)
22 Waye, M M Y, Winter, G, Wilkinson, A J & Fersht, A R. *EMBO J* **2**, 1827-1829 (1983)
23 Fersht, A R, Shindler, J. S & Tsui, W.-C *Biochemistry* **19**, 5520-5524 (1980)

LETTERS TO NATURE

New quasars with $z = 3.4$ and 3.7 and the surface density of very high redshift quasars

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It has been suggested that there is a sharp decline in the space density of quasars beyond redshifts $z \sim 3.5$[1]. We describe here the discovery on a UKST (UK Schmidt telescope) low-dispersion IIIaF objective prism plate of a QSO (quasistellar object) with $z = 3.7$, which is the second highest redshift QSO known and, moreover, lies in the same region in which a QSO with $z = 3.61$ has already been reported[2]. We also note a QSO with $z = 3.4$. Before these observations, only seven QSOs with $z \geq 3.4$ were known throughout the whole sky. When compared with the results of a search for QSOs with $2.7 < z < 3.3$ on the same plate, these observations do not support suggestions of a $z \sim 3.5$ cut-off in the QSO distribution[1], but rather support the suggestion of a steady decline from $z \sim 2$. We show here that deep searches over small areas of sky are unlikely to be successful in discovering QSOs at $z > 4$ and that such searches must be carried out over very large areas, even if only to a moderate limiting magnitude of $m(R) \sim 19$.

The UKST, in combination with the 44-arc min objective prism and IIIaJ plates, has proved a powerful tool for the detection of QSOs by their Lyα emission up to $z = 3.3$, a limit set by Lyα then passing beyond the red limit of the IIIaJ response at ~5,200 Å. This particular prism has usually been considered to have too low a dispersion to be used effectively with a IIIaF emulsion, which has a red limit at ~6,800 Å and can, in principle, detect QSOs up to $z \approx 4.5$. However, the discovery of the $z = 3.52$ QSO1159+123 (ref. 3) has shown that UKST IIIaF prism plates can be used to find high-z QSOs; the selection problems caused by irregularities in the spectral response of the

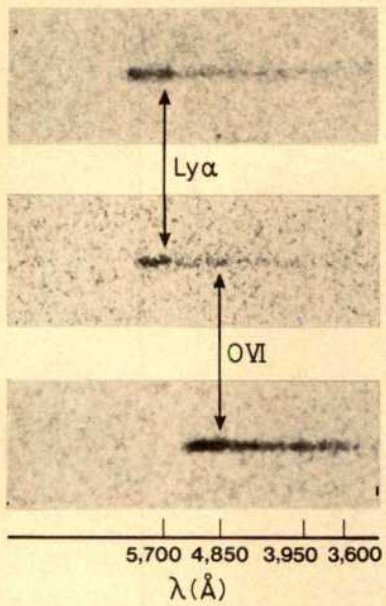


Fig. 1 Objective prism spectra of 0055−2659 reproduced by courtesy of the UK Schmidt Telescope Unit.

IIIaF emulsion can be minimized by using auxiliary IIaD and IIIaJ prism plates to check selected candidates. The rapid decrease of the prism dispersion with increasing wavelength produces in the majority of IIIaF prism spectra a strong enhancement of the continuum emission near their red limits. This makes emission lines above 6,000 Å difficult to distinguish and limits the maximum redshift attainable to $z \cong 3.9$. As QSOs with $z \leqslant 3.3$ are better found on IIIaJ plates, the useful range of the IIIaF plates is $3.3 \leqslant z \leqslant 3.9$.

The new QSOs were discovered on a set of high-quality plates centred on RA 00 h 53 min, dec. −28° 03′, the same field in which the $z = 3.61$ QSO DHM0054−284 (ref. 2) was found. Their positions, magnitudes and estimated redshifts are given in Table 1. These were the only new objects found in our search about which we were reasonably certain on the basis of the prism data alone, although we would certainly have included DHM0054−284 in Table 1 if it had not already been identified. The objective prism spectra of the $z = 3.7$ QSO0055−2659 are reproduced in Fig. 1 and illustrate how the IIaD and IIIaJ spectra are used to support the identification. The Lyα emission visible in both the IIIaF and IIaD spectra, the OVI emission visible in the IIaD and IIIaJ spectra and the heavy absorption in the IIIaJ continuum, all combine to support the identification as a high-z QSO. Our measurements of the various emission features are presented in Table 2 and the estimated redshifts are in such good agreement that we had no hesitation in confirming from the prism data alone the identity of 0055−2659 as a QSO with $z = 3.7 \pm 0.05$. Its strong blue continuum, extending to at least 800 Å in the rest frame, makes this bright QSO particularly suitable for ground-based absorption line studies and for satellite observations. The $z = 3.4$ QSO0042−2627, could also be identified with confidence. The emission line in the IIIaF spectrum is too strong to be an artefact of the emulsion response and it is just visible at the extreme limit of the IIIaJ spectrum.

Observations by A. Boksenberg (personal communication) on the 2.5-m Isaac Newton telescope on La Palma have already confirmed the estimated redshift for 0055−2659, and those by A. Boksenberg and W. L. W. Sargent (personal communication) on the Palomar 5-m telescope, that of 0042−2657. The measured redshifts are 3.67 and 3.4, respectively, which establishes 0055−2659 as second in redshift only to the $z = 3.78$ radio-selected QSO2000−330 (ref. 4) and as the highest redshift QSO selected by purely optical techniques. Of the nine known QSOs with $z \geqslant 3.4$, three have now been discovered in the 36 square degrees of one UKST field, a success rate that raises questions regarding the failure of previous high-z QSO surveys going to fainter magnitude limits.

The failure to find any QSOs with $z > 3.8$ in the earlier special searches over areas of only a few square degrees must be considered in light of the fact that even in the redshift range $3 < z \leqslant 3.3$ QSOs are not exactly common. Although even weak emission-line QSOs in this redshift range are readily found on Schmidt Telescope IIIaJ prism plates, and such plates covering several thousands of square degrees have now been searched, the latest QSO catalogue[5] lists only 38 in this redshift interval. As far as we know, the highest number of QSOs with $3 < z \leqslant 3.3$ on a single Schmidt plate has been found by C.H. and W. L. W. Sargent (unpublished data) on a UKST plate centred on RA 15 h 00 min, dec. +11° 00′. They have confirmed by slit spectroscopy a total of nine such QSOs with $B(J) < 20$, with only a few candidates remaining to be examined so far. This represents a decrease by a factor of at least 10 from the number of QSOs they found around $z = 2$ in the same redshift interval. Hazard[6] claims that this steep decrease cannot be attributed to a bias against the selection of the higher-z QSOs but corresponds to a decrease in the co-moving density of emission-line QSOs for $z \geqslant 2$. He further suggests that the failure to detect QSOs around $z = 4$ over small areas of sky, rather than implying a QSO cut-off at $z \sim 3.5$, represents an extrapolation of the observed decline between $z = 2$ and $z = 3$ to higher redshifts.

Assuming $m(J) - m(R) \approx 0.7$ for QSOs around $z = 3$ (see Fig. 1 in ref. (2)) and a rate of decrease of a factor of 10 per unit redshift interval, the extrapolation gives an expected six QSOs per UKST field with $m(R) < 19.3$ and $3.3 < z < 3.9$. The discovery of three QSOs in the 0053−2803 field with $m(R) \leqslant 18$ is consistent with this estimate, particularly as some of the $z > 3$ QSOs in the 1500+1100 field are weak emission line objects that would probably not have been detected on the IIIaF plate. The only surprise, perhaps, is that all are so bright, as, for the usual power law luminosity function, the majority would be expected to lie near the plate limit at about 19 mag; we have searched carefully for fainter candidates, but without success, apart from a possible weak line $z = 3.5$ QSO with $m(R) = 18.4$ at RA 01 h 01 min 15.7 s, dec. −26° 53′ 26″.

Any significant clustering of high-z QSOs or any difference in the redshift distribution in different regions of sky could invalidate the above comparison. We have, therefore, attempted a direct comparison of the QSO densities between $3.3 < z < 3.9$ and $z \sim 3$ by searching the IIIaF plate for QSOs with $2.7 < z < 3.3$ using the same procedures adopted in the search for the higher-redshift objects. Genuine QSOs with $z < 3.3$ can be unambiguously identified by their Lyα emission in the IIIaJ spectra. We did, indeed, find that not all the $z \sim 3$ QSOs that are recognizable on the IIIaJ plates can be found on the IIIaF plates, showing that our survey for objects with $z > 3.3$ is also probably incomplete. The search yielded a total of 11 QSOs with $2.7 < z < 3.3$. When compared with our results for $3.3 < z < 3.9$, this represents, for a magnitude-limited sample and for $\Delta z = +0.6$, a decrease in QSO density by a factor of 3, which is in satisfactory agreement

Table 1 Positions, magnitudes and estimated redshifts for new QSOs

	z	RA (h	min	s)	dec. (°	′	″)	$m(R)$*	$M(R)$†
0042−2627	3.4	00	42	06.2	−26	27	43	~17	~−31.7
0055−2659	3.7	00	55	32.5	−26	59	25	17.2	−31.8
(DHM0054−284									−31.2)

* UKST R IIIaF emulsion, RG 630 filter (measured on Cambridge APM).
† Calculated assuming a Hubble constant, $H_0 = 50$ km s^{-1} Mpc^{-1}, deceleration in parameter $q_0 = 0$.

Table 2 Emission features of QSO0055-2659

Emulsion	λ (Å)	Identification	z
IIIaF	5,700	Lyman-α	3.70
IIaD	5,690	Lyman-α	3.69
IIIaJ	4,875	OVI	3.71
		Adopted redshift 3.70 ± 0.05	

with the decrease by a factor of ≥ 10, between $z = 2$ and $z = 3$ found on the 1500+1100 plate. These results are more consistent with a steady decline in the QSO density above $z > 2$ than with a cut-off in the QSO distribution at $z \sim 3.5$. This could imply that the birth of the majority of QSOs occurred at $z \sim 2$ and could have important implications regarding the epoch of galaxy formation and the rate of growth of the massive galactic nuclei that are generally believed to be the primary QSO energy sources.

Our results, relating both to the fall in the surface density of QSOs with increasing redshift and to the apparent lack of a strong magnitude dependence in the number of high-z QSOs, are consistent with the type of combined luminosity and density evolution proposed by Koo[7]. According to Koo, the luminosity function at $z = 2.15$ corresponds to a steep power law dependence at the bright end with the QSO density rising rapidly with decreasing brightness up to $M_B = -28$, where there is a transition to a much flatter distribution for the fainter objects. With increasing redshift the basic form of the distribution remains unaltered, but it is displaced in the log ρ/M plane so that the turn-over point shifts both to brighter absolute magnitudes and to lower densities. For sufficiently high redshifts the observed QSOs will be dominated by those lying in the flat region between the turn-off point and the absolute magnitude corresponding to the limiting apparent magnitude. The occurrence of a bright high-z QSO in a given field is thus as probable as a QSO near the limiting magnitude, although, in practice, the former is the one more likely to be selected; that all known high-z QSOs are relatively bright is therefore no accident. In a magnitude-limited sample, the number of QSOs in a given redshift interval will decrease with increasing redshift at a rate approximately corresponding to the degree of density evolution, but modified by the different rates of displacement of the turn-off point and the limiting magnitude; if there is a maximum QSO luminosity, the numbers will decrease more rapidly when it comes into effect.

It is easy to show, by a similar extrapolation to that used earlier, that the expected number of QSOs to $m(R) \sim 21$ with $4 < z < 5$ is less than 0.1 per square degree, a figure quite consistent with the reported null detections over an area of 5 square degrees[1]. Furthermore, this figure is only weakly dependent on the magnitude limit and about half of the QSOs will be brighter than 19 mag. Deeper surveys cannot be used to compensate for a drastically reduced search area and it follows that searches for very high-z QSOs, carried out over small areas of sky, have a very low probability of success. Searches over very large areas, even if only to a moderate limiting magnitude of $m(R) \sim 19$, offer the only realistic hope.

We have demonstrated the usefulness of the UKST low-dispersion IIIaF objective prism plates in high redshift surveys, at least up to $z \sim 3.7$. The extension to $z > 4$ will probably require the use of the new higher-dispersion prism with appropriate filters or the use of broad-band colours. The strong Lyα emission of all presently known high-z QSOs has at least removed the worry that increasing absorption due to intervening gas or dust might prevent their discovery when using slitless spectroscopy techniques. Finally, the observations presented here should encourage more active searches for these objects which provide the only means we have at present for probing the early history of the Universe.

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1. Osmer, P. S. *Astrophys. J.* **253**, 28–37 (1982).
2. Shanks, T., Fong, R. & Boyle, B. J. *Nature* **303**, 156–158 (1983).
3. Hazard, C. *et al. Mon. Not. R. astr. Soc.* **211**, 45p–50p (1984).
4. Peterson, B., Savage, A., Jauncey, D. & Wright, A. *Astrophys. J.* **260**, L27–L29 (1982).
5. Veron-Cetty, M.-P. & Veron, P. *ESO Sci. Rep.* **1** (1984).
6. Hazard, C. in *Proc. Manchester Conf. Active Galactic Nuclei* (in the press).
7. Koo, D. C. *Proc. 24th Liège IAU Colloquium*, 240–244 (1983).

Infrared Seyferts: a new population of active galaxies?

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A Seyfert spectrum is one of the most important indicators of nuclear activity in galaxies. It is characterized by broad emission lines with high ionization. Most known Seyferts have been found indirectly, for example, by the presence of a strong ultraviolet continuum (Markarian galaxies) or of intense radio emission (broad/narrow-line radio galaxies). Here, we present a new method for finding hitherto unknown Seyferts based on their mid-infrared spectra and we show using data from the Infrared Astronomical Satellite (IRAS) that this method has a success rate of ~70%. One Seyfert, so discovered, contains the most luminous infrared nucleus known so far. X-ray surveys are also an efficient means of finding new Seyferts and QSOs (quasistellar objects)[1,2], but tend to reveal samples at considerably higher redshifts and lower space density. We believe that 'infrared' Seyferts may soon outnumber those detected by other methods.

IRAS[3] has detected many thousands of galaxies, principally at wavelengths of 60 and 100 μm. Most galaxies detected in flux-limited samples at these wavelengths[4,5] have relatively steep infrared spectra with indices $\alpha \sim -2.5$ (defined by $S_\nu \propto \nu^\alpha$, where S_ν is the flux density at frequency ν). This cold infrared emission (~ 30 K) is believed to be produced in the disks of spiral and interacting galaxies[5–7].

On the other hand, for most known cases of infrared emission from the nuclear region of active galaxies, the spectrum is significantly flatter in the infrared. For example, NGC1068 has a 250 K mid-infrared component whose spectrum peaks near 30 μm[8–11] and which originates from a region ~60 pc in size. A similar component is observed in 3C390.3[12]. Quasars also have relatively flat infrared spectra[13].

These considerations prompted us to investigate whether a relatively flat infrared spectrum may be an indicator of nuclear activity. We selected a subsample of 'warm' objects from the partially completed IRAS survey, all at high galactic latitude ($|b| > 20°$) and with spectral indices between 25 and 60 μm of $-1.25 < \alpha < -0.5$. These wavelengths were chosen as the most efficient for indicating flat-spectrum nuclear infrared emission. A cold galactic disk would dominate the 100–60-μm spectrum, possibly masking a warm nuclear component.

The IRAS sources so defined were identified using the Palomar Sky Survey and ESO/SRC (European Southern Observatory/Science Research Council) plates. After excluding objects that were clearly galactic (stars, small H II regions, planetary nebula), the remainder (54 objects) were published in IRAS Circular 11 as a list of candidate active nuclei[14].

Spectra were taken of 40 of these objects (Table 1). The observations were made in photometric weather between 7 and 9 March 1984 with the Image Dissector Scanner (IDS) on the Boller and Chivens spectrograph at the 3.6-m ESO telescope at La Silla with a dispersion of 172 Å mm^{-1}. This resulted in a wavelength coverage of 3,800–7,000 Å, with a resolution of 11-Å FWHM. The exposure times for the objects discussed in this letter was 10 min. Wavelength calibration, flatfielding and flux calibration were carried out in the standard way with the ESO IHAP system (Image Handling and Processing), using the white dwarfs W485 and L745−46A as standard stars.

Our results are summarized in Table 1. In classifying the optical spectra, we adopt the scheme of Baldwin *et al.*[15] in which

Table 1 Results of the spectroscopic observations

Name	Infrared spectrum	Redshift	[OIII] linewidth FWHM (km s^{-1})	Optical spectrum
0513−235P11		0.035:		No emission lines
0521−122P11	F1	0.0494	640	Seyfert I
0531−206P11	F2	0.0393		Strong Hα
0556−348P11		0.032:		No emission lines in both components
0611−326P11	F1	0.0500	630	Seyfert II
0815+035P11		0.030:		Strong Hα
0818+033P11		0.009:		No emission lines
1036−190P11		—		No emission lines
1051−273P11	F1	0.1606	720	Seyfert II
1105−115P11	F1	0.0545	780	Seyfert II
1119+045P11	F1	0.0386		Strong Hα
1121−281P11	F1	0.0136	750	Seyfert II
1246−111P11	F1	0.0483	950	Seyfert II
1249−131P11		0.0138	370	Seyfert I
1304−234P11	F1	0.0092	550	Seyfert II
1305−241P11	F1	0.0137	580	Seyfert II
1316−242P11		0.0322		Weak Hα (N-W component)
		0.035:		Strong Hα (S-E component)
1319−164P11	F1	0.0167	1250	Seyfert II
1320−342P11		—		No emission lines
1329+022P11	F2	0.0863	1120	Seyfert II
1331−301P11	F2	0.0344		Strong Hα
1331−234P11		0.0086	530	Seyfert II
1331−231P11		0.0446	870	Seyfert II (E-component has no emission lines)
1333−340P11	F1	0.0077	370	Seyfert I
1354−203P11	F2	0.0360		Strong Hα (S component has no emission lines)
1356−188P11		0.0315		Strong Hα
1402−316P11		0.093:		No emission lines
1404+012P11	F2	—		No emission lines
1423−116P11		0.0419	<200	Nuclear HII region
1428−030P11		0.0436		Strong Hα(N component)
		0.044:		Weak Hα (S component)
1431−326P11	F1	0.0255	690	Seyfert II
1444−219P11	F2	0.0107		Strong Hα
1458−222P11		0.045:		Weak Hα (W component has no emission lines)
1509−211P11	F1	0.0442	520	Seyfert I
1524+007P11	F1	0.0506	480	Seyfert II
1548−037P11	F1	0.0301	620	Seyfert II
1618+068P11	F2	—		No emission lines
1832−594P11	F1	0.0192	950	Seyfert II
1840−624P11	F1	0.0135	480	Seyfert I
1844−532P11		0.0185	280	Seyfert II

Column 1 lists the IRAS designation as given in IRAS Circular 11. Column 2 codes for the shape of the infrared spectrum ($\alpha > -1.25$): F1 marks a flat spectrum between 25 and 60 μm; F2 denotes that the 25-μm flux is unreliable, but that the spectrum is flat between 60 and 100 μm. The measured redshifts are given in column 3 (the error is smaller than 0.001, except for those marked ':'. The corrected [OIII] linewidths (FWHM deconvolved with the instrumental response) are given in column 4 and the typical uncertainty is 60 km s^{-1}. Column 5 gives the type of optical spectrum and information about the number of optical counterparts possibly associated with the infrared sources.

Seyfert galaxies have high ionization with [OIII]λ5007/$H\beta > 6$ and [NII]λ6584/Hα > 0.4. All the spectra defined in this way have broad resolved [OIII] lines >250 km s^{-1} full-width to half maximum (FWHM), also typical of Seyferts[16–18]. Although higher resolution spectra are clearly necessary, the results obtained up to now suggest that on average the widths of the forbidden lines are substantially larger (57% broader than 600 km s^{-1}) than in previous surveys[19] (15% broader than 600 km s^{-1}), which may be an effect of the large fraction of Seyfert IIs in this sample, as they are known to have broad forbidden lines[16].

One of these infrared Seyferts (Fig. 1) is IRAS1051−273P11. Our measured redshift of 0.161 combined with the IRAS 25-μm

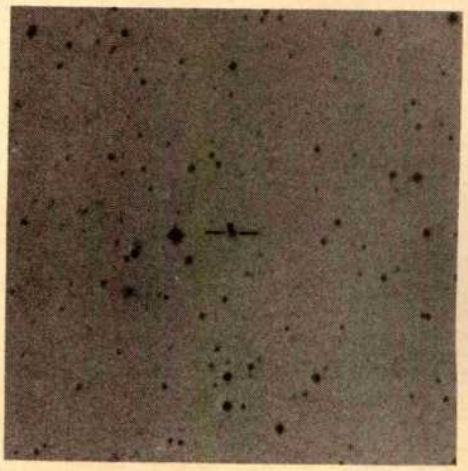


Fig. 1 A reproduction of the ESO/SRC J-plate of IRAS1051−273P11. North is up, east to the left; the picture encompasses 5×5 arc min on the sky. Close to the north of this Seyfert galaxy is a star.

flux density of 0.4 Jy results in a 25-μm luminosity of $10^{25.3}$ W Hz^{-1}, which is an order of magnitude larger than that of the mid-infrared component of either NGC1068 or 3C390.3, making it the most luminous Seyfert galaxy known in this wavelength range. If the 25-μm flux is thermal in origin, arguments analogous to that used for NGC1068[11] would require heating by an ultraviolet source of 2×10^{12} L_0.

In Table 1, 24 objects have reliably-determined flat infrared spectra between either 60 and 25 μm or between 60 and 100 μm. Of these flat-spectrum objects 17 (that is, 71%) have Seyfert spectra, which should be compared with a Seyfert rate of ~10% for Markarian objects. Hence a flat infrared spectrum is a remarkably efficient predictor of the Seyfert phenomenon and a preliminary analysis of IRAS circular 16 (ref. 20), which contains sources with similar infrared colours, seems to confirm this picture. Also, the IRAS source 0421+040P06[21,22], which has a 25-60-μm spectral index within our selected range, has been found recently to have a Seyfert-type spectrum.

Extrapolation of our results to the all-sky survey indicates that the IRAS database contains several thousand Seyferts, compared with ~330 Seyferts previously known[23]. Taking the redshifts of the 21 Seyferts in Table 1 and assuming a Hubble constant of 75 km s^{-1} Mpc^{-1}, the space density of the infrared Seyferts, determined using the V/V_{max} method, is 1.7×10^{-4} Mpc^{-3}, 2.5 times greater than values based on the Markarian Seyferts[24]. As no correction was made for incompleteness, this should be viewed as a lower limit. The fractional error based on this sample is 40%, but combination with recent new data yields the same figure with a 25% uncertainty.

Seyferts found through X-ray surveys are quite distinct in their properties from the infrared Seyferts; the space density of the X-ray objects is more than an order of magnitude lower and they are on average 10 times further away. Indeed a large fraction of them may more properly be called QSOs.

How do the infrared Seyferts differ from the Markarian Seyferts and why have they not been discovered before? The possibility that, using infrared criteria, we select more distant galaxies than does the Markarian method is improbable as there is no significant difference in the redshift distribution of our sample compared with the Markarian one[24] and both have median redshifts of 0.03. It is more probable that the infrared Seyferts are systematically fainter in the optical than the Markarian Seyferts[24]. The Markarian catalogue becomes incomplete below an apparent magnitude of 15.5; although there is considerable uncertainty in the brightness of the infrared Seyferts, our initial estimates indicate that several are fainter than this magnitude.

It is also not unexpected that a large number of infrared Seyferts would be missed by the Markarian catalogue. The same dust that is presumably responsible for the infrared emission would significantly redden the non-thermal visible light from

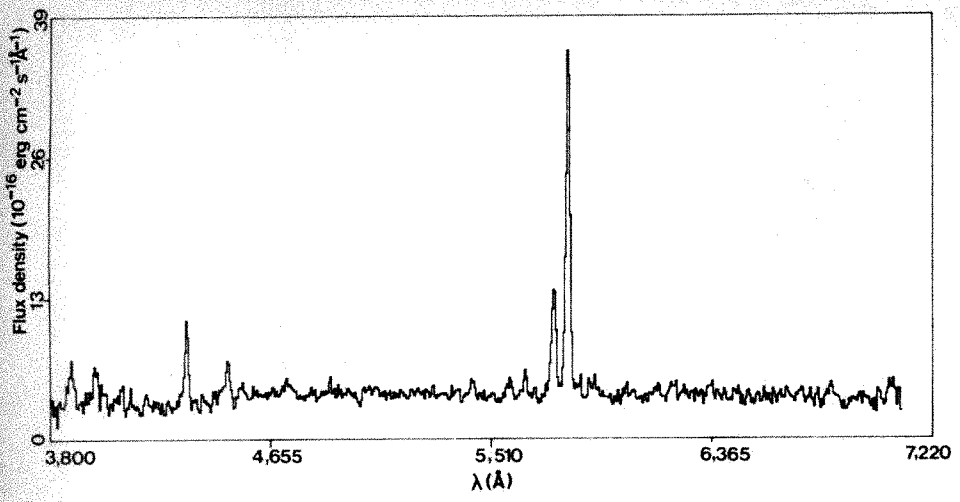


Fig. 2 The spectrum of IRAS1051−273P11 taken with the 3.6-m telescope at La Silla. A correction for the instrumental sensitivity has been applied, which tends to increase the noise on the edges of the spectrum. Note that Hα is redshifted out of the wavelength range.

the nucleus, thereby reducing or eliminating the ultraviolet excess required for inclusion in the Markarian survey. Obscuration by dust outside the nucleus could also significantly affect the detectability. Hence the Markarian (ultraviolet) and IRAS (infrared) techniques are complementary indicators of the Seyfert phenomenon.

We show here that an infrared survey can be a highly efficient means of discovering new Seyferts. The nature of the objects selected in this way and their relationship with other active galaxies will be studied further. Comparison of the infrared properties with the intensities and profiles of the narrow emission lines will give unique information about the role of dust in the narrow line region.

A study of the visible morphology and the radio properties of the infrared Seyferts is of particular interest. These Seyferts may be a link between optically-selected Seyferts, which are associated predominantly with spirals, and the radio-selected ones, which are in elliptical galaxies.

Finally, in addition to the all-sky survey discussed here, a deeper survey of limited regions of the sky was carried out by IRAS. This survey should reach factors of 5–15 times deeper than the all-sky survey and contain several thousand sources and should enable infrared Seyferts to be detected out to redshifts of >0.5 where the effects of cosmological evolution are believed to play an important role.

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1. Stocke, J. T. *et al. Astrophys. J.* **273,** 458–477 (1983).
2. Gioia, I. M. *et al. Astrophys. J.* **283,** 495–511 (1984).
3. Neugebauer, G. *et al. Astrophys. J. Lett.* **278,** L1–L6 (1984).
4. Soifer, B. T. *et al. Astrophys. J. Lett.* **278,** L71–L74 (1984).
5. Young, E. *et al. Astropys. J. Lett.* **278,** L75–L78 (1984).
6. Habing, H. J. *et al. Astrophys. J. Lett.* **278,** L59–L62 (1984).
7. De Jong, T. *et al. Astrophys. J. Lett.* **278,** L67–L70 (1984).
8. Rieke, G. H. & Low, F. J. *Astrophys. J. Lett.* **199,** L13–L17 (1975).
9. Simon, T. & Dyck, H. M. *Mon. Not. R. astr. Soc.* **172,** 19P–21P (1975).
10. Jameson, R. F., Longmore, A. J., McLinn, J. A. & Woolf, N. J. *Astrophys. J. Lett.* **187,** L109 (1974).
11. Jones, T. W., Leung, C. M., Gould, R. J. & Stein, W. A. *Astrophys. J.* **212,** 51–59 (1977).
12. Miley, G. K. *et al. Astrophys. J. Lett.* **278,** L79–L81 (1984).
13. Neugebauer, G. *et al. Astrophys. J. Lett.* **278,** L83–L85 (1984).
14. *IRAS Circ. 11 Astr. Astrophys.* **134,** C5 (1984).
15. Baldwin, J. A., Phillips, M. M. & Terlevich, R. *Publs astr. Soc. Pacif.* **93,** 5–19 (1981).
16. Feldmann, F. R., Weedman, D. W., Balzano, V. A. & Ramsey, L. W. *Astrophys. J.* **256,** 427–434 (1982).
17. Véron, M. P. *Astr. Astrophys.* **100,** 12–19 (1981).
18. Shuder, J. M. & Osterbrock, D. E. *Astrophys. J.* **250,** 55–65 (1981).
19. Heckman, T. M., Miley, G. K. & Green, R. F. *Astrophys. J.* **281,** 525–534 (1984).
20. *IRAS Circ. 16 Astr. Astrophys.* (in the press).
21. *IRAS Circ. 6 Astr. Astrophys.* **131,** C1 (1984).
22. Beichman, C. *et al. Astrophys. J. Lett.* (in the press).
23. Véron-Cetty, M. P. & Véron, P. *European Southern Observatory Sci. Rep.* **1,** (1984).
24. Meurs, E. J. A. & Wilson, A. S. *Astr. Astrophys.* (in the press).

On the binary nature of cosmic γ-ray burst sources

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The basic nature of cosmic γ-ray burst sources[1] has remained a mystery. Schaefer[2] and Schaefer *et al.*[3] recently discovered three images on archival photographic plates probably due to optical flashes from the error boxes of three γ-ray burst sources. This discovery opens up the possibility of ground-based optical studies of these objects (see, for example, ref. 4) and provides an important new set of data that can constrain models significantly (see, for example, ref. 5 and references therein) for these intriguing sources. London and Cominsky[6] have considered a model for the optical flashes, wherein γ-ray bursts are emitted by a collapsed object in a close-binary stellar system and a small fraction of the γ radiation is reprocessed into optical radiation in the surface layers of the companion star (see refs 7 and 8 for possible alternative explanations). Based on their own estimates of the time, τ, required to reprocess the γ radiation into optical light, London and Cominsky concluded that a binary model would not account for the observed optical flashes. They considered, however, only main-sequence stars and hydrogen-depleted degenerate dwarfs as possible companion stars. We reconsider here the binary model and conclude that it is viable. In particular, under the assumption that the optical flashes were produced by γ-ray bursts of about the same intensity as those observed, we find that nearby (≲100 pc) binary systems with secondaries whose masses are less than ~0.06 $M_\odot$ can fit all the observational constraints for the three optical/γ-ray pair events (hereafter 1928/1978, 1944/1979 and 1901/1979; see Table 1).

We have considered a wide range of parameters for the binary systems in which the optical flashes may be produced. Here we have chosen specifically a sequence of models that could plausibly represent the evolution of a compact binary during the phase when the mass of the secondary is reduced (by mass transfer) from ~1 $M_\odot$ to almost planetary values. We adapted the results from a binary evolutionary sequence calculated by Rappaport, Verbunt and Joss[9], in which the initial binary consists of a collapsed star of mass 1.2 $M_\odot$ orbiting a 1.0 $M_\odot$

main-sequence dwarf; the early evolution is dominated by the loss of angular momentum due to magnetic braking, whereas the later evolution is dominated by the emission of gravitational radiation (see model CMB 4 in ref. 9 for details). For the present work, we took the final mass of the primary to be no greater than 1.4 $M_\odot$ and adjusted the orbital separation accordingly. The evolution of the mass, radius and effective temperature of the companion star in this evolutionary sequence, as well as the orbital period and mass transfer rate, is shown in Fig. 1. As the mass of the secondary, M_c, decreases from 1.0 $M_\odot$ to 0.1 $M_\odot$, its properties remain close to those of a main-sequence star of the same mass. For $0.03 \leq M_c \leq 0.1\ M_\odot$, the secondary star is out of thermal equilibrium and evolving toward a degenerate state; for $M_c \leq 0.03\ M_\odot$, the internal properties of the secondary are essentially those of a completely degenerate star. The results described below are not especially sensitive to the details of this evolutionary scenario; the calculated optical fluences depend primarily on the current orbital separation and the radius and effective temperature of the secondary.

We do not attempt here to model the γ-ray burst emission mechanism, but assume merely that γ radiation is emitted isotropically by the collapsed star. For each of the binary models, we consider γ-ray burst energies, E_γ, in the range of 10^{35}–10^{39} erg and use an approximate expression for the average γ-ray fluence, F_{γ_c}, that is absorbed over the facing hemisphere of the companion star

$$F_{\gamma_c} = \frac{E_\gamma(1-\alpha)\{1-[1-(R_c/a)^2]^{1/2}\}}{4\pi R_c^2} \simeq \frac{E_\gamma}{8\pi a^2} \simeq 1.6\times10^{14}\left[\frac{E_\gamma}{10^{37}\ \mathrm{erg}}\right] \times\left[\frac{a}{5\times10^{10}\ \mathrm{cm}}\right]^{-2} \mathrm{erg\ cm^{-2}} \quad (1)$$

where α is the γ-ray albedo (taken to be 0.2), R_c is the radius of the secondary and a is the orbital separation.

The γ radiation, which penetrates the surface layers of the companion star, is thermalized and re-radiated as optical light (and possibly also as infrared and ultraviolet radiation). We have carried out Monte Carlo calculations to determine the column density beneath the stellar surface at which the burst energy is deposited and find that for typical burst spectra, the amount of energy deposited falls off approximately exponentially, with an e-folding column density of ~8–10 g cm^{-2} (see also ref. 6).

London and Cominsky[6] have estimated the reprocessing time, τ, and find that for γ-ray fluences (at the companion) in the range $10^{12.4} < F_{\gamma_c} < 10^{14.8}$ erg cm^{-2}, the value of τ can greatly exceed the duration of the γ-ray burst. Their estimates, however, are only of order of magnitude accuracy and neglect the effects of the hydrodynamic expansion of the envelope of the secondary. Therefore, we consider here a rather wide range of reprocessing times of 5–500 s. The lower limit is set by the typical duration of the γ-ray burst events[10,11], whereas the upper limit is set by the lack of trailing of two of the detected optical images[2,3] (see Table 1). For simplicity, we assume that during the optical flash the heated hemisphere of the companion star radiates uniformly for a time τ at a constant temperature, $T_b = [F_{\gamma_c}/\sigma\tau + T_{qui}^4]^{1/4}$ (see also ref. 6), where σ is the Stefan-Boltzmann constant and T_{qui} is the effective temperature of the companion star in quiescence between γ-ray burst events.

We calculated the optical fluence at the Earth in a 1,000-Å-wide band centred at 4,350 Å (a blue band that approximates the response of the archival plates on which the optical flashes were discovered[2,3]) for each model in our evolutionary sequence and for each of several X-ray burst energies within the range prescribed above. For simplicity, we assumed a typical aspect angle of 45° between the line of sight and the line connecting the centres of the binary components. We also computed the distance to the source implied by the measured γ-ray fluence of each particular event at the Earth (see Table 1) and the assumed γ-ray burst energy, and the quiescent visual magnitude m_{qui} of the companion star. We require that $m_{qui} > 24$ for a model to be consistent with the lack of any prominent optical candidates[12–15] in the error boxes of the three γ-ray burst sources that have been associated with optical flashes[2,3].

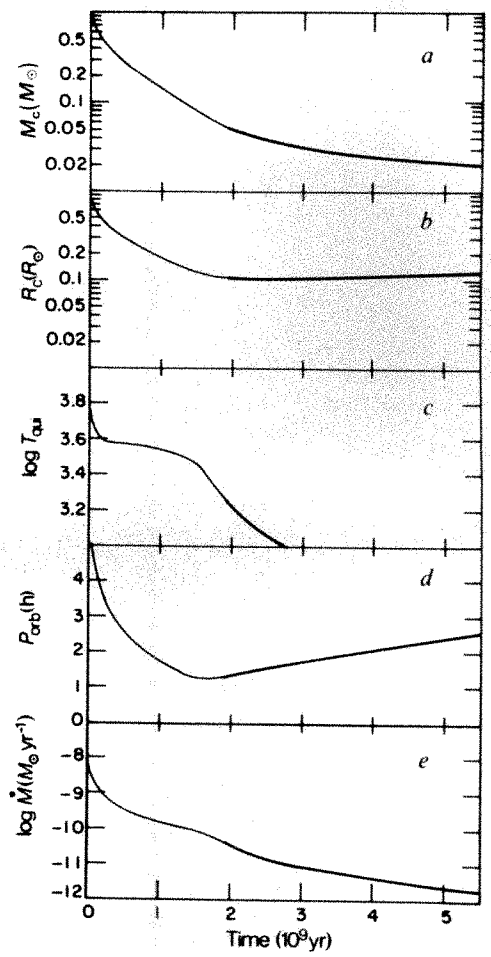


Fig. 1 Evolution of a compact binary system under the influence of magnetic braking and gravitational radiation (adapted from model CMB4 of ref. 9). The initial binary system consists of a 1.0 $M_\odot$ main-sequence dwarf companion and a 1.2 $M_\odot$ collapsed star in a 6.3-h orbit. The quantities plotted as functions of elapsed time in frames *a*–*e* are the mass, radius and quiescent effective temperature of the secondary star, the orbital period and the rate of mass loss from the secondary, respectively. The heavy portion of each curve corresponds to binary models which yield optical flashes (due to the reprocessing of γ-radiation) with properties similar to those observed[2,3]. The heavy portion of the effective temperature curve is qualitatively correct, but its quantitative details are highly uncertain.

We show sample results of our calculations in Fig. 2 for the 1928/1978 source (results for the 1944/1979 and 1901/1979 sources being similar). The γ-ray fluence of the 1978 event was ~3×10^{-4} erg cm^{-2} s^{-1}, whereas the blue magnitude of the optical flash on the archival plate was 10.2 (ref. 2). This corresponds to a blue fluence, F_{opt}, of ~$1{\cdot}4\times10^{-6}\ \mathcal{R}$ erg cm^{-2}, where $\mathcal{R}$ is a correction factor that takes into account the reciprocity failure[16] of the photographic emulsion. If the entire optical event transpired during the exposure of the plate, we obtain (from data presented in ref. 16) $\mathcal{R} \simeq (\tau/T_{exp})^{0.18}$, where T_{exp}, the exposure time of the plate, is 2,700 s for the plate on which the 1928 optical flash was discovered.

The results in Fig. 2 show the predicted values of F_{opt} as a function of the assumed γ-ray burst energy. Each burst energy corresponds to a unique distance to the source that generated the 19 November 1978 event if the model is to account for the measured γ-ray fluence. We show the results for binary models with several different values of M_c and $\tau_\odot$. (Our estimates for

Table 1 Properties of optical flashes from γ-ray burst sources*

Event pair name	1928/1978	1944/1979	1901/1979
γ-Ray burst source	19 Nov. 1978 GRB	13 Jan. 1979 GRB	5 Nov. 1979 GRB
γ-Ray fluence (erg cm^{-2})‡	3.2×10^{-4}	1.1×10^{-4}	1.3×10^{-5}
Depth of optical flash	17 Nov. 1928	19 Feb. 1944	4 Oct. 1901
Blue magnitude of flash	10.2	12.1	12.6†
Optical fluence (erg cm^{-2})§	4.7×10^{-7}	1.4×10^{-7}	1.6×10^{-8}
Plate exposure (s)	2,700	5,400	660
Trailing limit (s)‖	600	—	200

* The data are taken from refs 2 and 3.
† See ref. 16.
‡ For $E \geq 30$ keV
§ In a 1,000-Å band centred at 4,350 Å, for an assumed value of $\tau = 5$ s.
‖ Limit on the duration of the optical flash derived from the lack of trailing of the observed images.

F_{opt} are effectively lower limits, as the presence of a disk or ring of matter in the binary stellar system would probably increase the total blue fluence resulting from reprocessing.) The measured value of F_{opt}, which varies by up to a factor of two for different choices of τ because of the variation of the reciprocity correction, is also indicated.

We consider the agreement between the model calculations and the observations to be excellent when the following sources of error are taken into account: (1) the uncertainties in establishing the apparent magnitude of the optical flash (including uncertainties in reciprocity corrections for old emulsions[16] and uncertainties in comparing the untrailed flash image with trailed reference-star images[16]); (2) the uncertainty in the measured γ-ray fluence; (3) the unknown difference between the strength of the γ-ray burst that produced the 1928 optical flash and the observed γ-ray burst of 1978. In the light of these uncertainties, we consider agreement to within a factor of ~5 between the predicted and measured optical fluences to be an acceptable fit (see Fig. 2). In fact, as is evident from Fig. 2, there are a number of models with $M_c \leq 0.05\ M_\odot$ for which the predicted optical fluence is within a factor of ~2-3 of the measured fluence. Binary models providing acceptable fits are shown as heavy curves in Fig. 1. For each value of τ and M_c, the best-fit model roughly corresponds to the γ-ray energy that yields $T_b \simeq 8{,}500$ K and thereby maximizes the amount of reprocessed radiation in the blue band.

From the results shown in Fig. 2, we conclude that the proposed model for producing the optical flashes by reprocessing γ radiation on the surface of a binary stellar companion is viable. London and Cominsky[6] reached the opposite conclusion because they did not fully consider extremely-low-mass hydrogen-rich secondary stars. We find that the most favourable models for the 1928/1978 event are those with $M_c \leq 0.05\ M_\odot$, $T_{qui} \leq 1{,}800$ K, $E_\gamma \leq 10^{38}$ erg and distances $d \leq 50$ pc. Values of E_γ and d less than $\sim 10^{35}$ erg and ~2 pc, respectively, are allowed by the binary model, but are excluded by considerations (discussed below) that are based on the Oort limit and the diffuse cosmic X-ray background.

The same type of calculation as that represented in Fig. 2 was repeated for the 1944/1979 and 1901/1979 events. The binary models best able to account for these events are essentially the same as for the 1928/1978 event (Fig. 1). Acceptable distances are ≤100 and ≤250 pc for the 1944/1979 and 1901/1979 events, respectively.

Distances for these sources of ≤2 pc are excluded because the mass density of such systems would then exceed the Oort limit on the local density of matter in the solar neighbourhood (~0.15 $M_\odot$ pc^{-3}; see, for example, refs 12, 17 and 18). Moreover, if (1) the mean distance between sources is ≤10 pc, (2) the systems have neutron-star primaries, (3) these systems were low-mass X-ray binaries at an earlier evolutionary phase[19] and if (4) the number of such systems in our Galaxy is typical of other galaxies, then the total X-ray emission from such systems in distant galaxies would dominate the diffuse X-ray background[20].

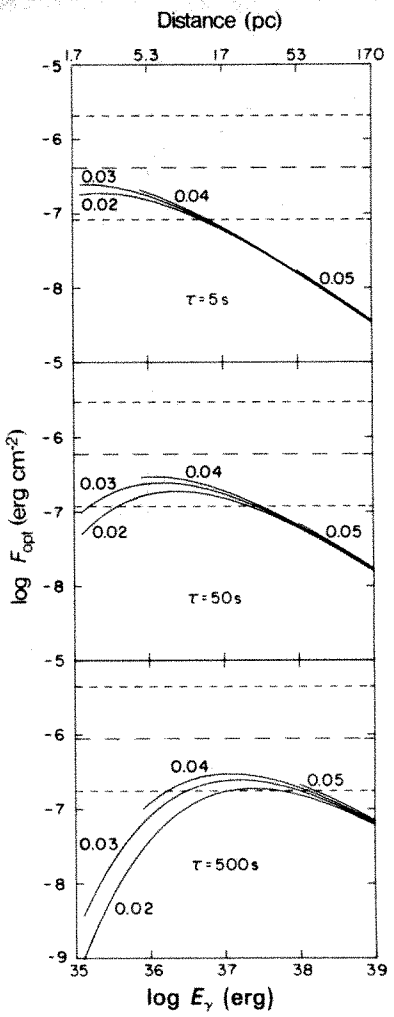


Fig. 2 Calculated optical fluences, F_{opt}, at the Earth, in a 1,000-Å band centred at 4,350 Å. The calculations are carried out for a γ-ray fluence at the Earth of 3×10^{-4} erg cm^{-2} and for a range of binary models, assumed γ-ray burst energies, E_γ, and reprocessing times, τ. The binary model is completely specified by the mass of the secondary, M_c (see Fig. 1); sample results are shown for $M_c = 0.02$, 0.03, 0.04 and 0.05 $M_\odot$. The curve for each value of M_c terminates at the point where the companion star would be visible in quiescence ($m_{qui} < 24$; see text). The long dashed line in each frame is the measured optical fluence[2] of the 1928/1978 event (see Table 1). Note that this quantity attains a somewhat different value for each of the assumed reprocessing times because of variations in the correction for reciprocity failure. The set of short dashed curves in each frame indicates a range of a factor of 5 around the measured optical fluence, within which the model fits are considered to be acceptable.

The need for more detailed calculations of the γ-ray reprocessing times is evident from Fig. 2. If we knew τ more reliably (for a given model of the secondary star and a given γ-ray fluence at the secondary), a much more restrictive range of distances to each source could be obtained.

If the reprocessing model is the correct explanation of the optical flashes from γ-ray burst sources, we have already established some very specific properties of these sources. They are binaries containing a low-mass cool secondary star, in orbit with a collapsed star (most probably a neutron star or degenerate dwarf) that is the source of the γ-ray bursts. The particular systems that produced the observed optical flashes may be relatively close neighbours of the Solar System; this inference is consistent with the unusually large measured γ-ray fluences[10,11] of the 1928/1978 and 1944/1979 events.

The type of binary system we have invoked, although yielding apparently correct γ-ray reprocessing properties, presents a difficulty that must be resolved if the model is to be viable. The mass-loss rates from the secondary lie in the range of $\sim 0.2\text{-}3 \times 10^{-11}\ M_\odot\ \mathrm{yr}^{-1}$ (Fig. 1). If this matter were accreted onto a $\sim 1\ M_\odot$ collapsed star and emitted isotropically in the X-ray range, the flux at the Earth would be

$$F_x \simeq 7 \times 10^9 \left[\frac{\dot{M}}{10^{-12} M_\odot\ \mathrm{yr}^{-1}}\right]\left[\frac{D}{100\ \mathrm{pc}}\right]^{-2} \times \left[\frac{R}{10^6\ \mathrm{cm}}\right]^{-1} \mathrm{erg\ cm^{-2}\ s^{-1}} \quad (2)$$

where R is the radius of the collapsed star. The measured X-ray flux from the 19 November 1978 source in quiescence is $\sim 10^{-13}$ erg cm^{-2} s^{-1} (ref. 21), which is ~ 5 orders of magnitude below the level implied by equation (2) for a neutron star, at a distance of $\leqslant 100$ pc, accreting at $\geqslant 10^{-12}\ M_\odot\ \mathrm{yr}^{-1}$. This dilemma may be resolved if there is a reduction or cessation in the rate at which matter is steadily accreted onto the collapsed star when the mass-loss rate from the secondary falls below some critical value. (We note that this picture may support some theoretical ideas concerning the nature of γ-ray bursts[22].) It is also possible that the accretion-driven luminosity will be emitted at ultraviolet or very soft X-ray energies when the accretion rate is very low.

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1. Klebesadel, R. W., Strong, I. B. & Olson, R. A. *Astrophys. J. Lett.* **182**, L85-L88 (1973).
2. Schaefer, B. E. *Nature* **294**, 722-724 (1981).
3. Schaefer, B. E. *et al. Astrophys. J. Lett.* **286**, L1-L4 (1984).
4. Ricker, G. R., Doty, J. P., Vallerga, J. V. & Vanderspek, R. K. *Soc. Photo-Opt. Instrum. Engr.* **445**, 370 (1984).
5. *High-Energy Transients in Astrophysics* (ed. Woosley, S. E.) (American Institute of Physics, New York, 1984).
6. London, R. A. & Cominsky, L. R. *Astrophys. J. Lett.* **275**, L59-L63 (1983).
7. Woosley, S. E. in *High-Energy Transients in Astrophysics* (ed. Woosley, S. E.) 485 (American Institute of Physics, New York, 1984).
8. Katz, J. *Astrophys. Lett.* (in the press).
9. Rappaport, S., Verbunt, F. & Joss, P. C. *Astrophys. J.* **275**, 713-731 (1983).
10. Mazets, E. P. *et al. Astrophys. Space Sci.* **80**, 3-83 (1981).
11. Mazets, E. P. *et al. Astrophys. Space Sci.* **80**, 119-143 (1981).
12. Schaefer, B. E. & Ricker, G. R. *Nature* **302**, 43-45 (1983).
13. Schaefer, B. E., Seitzer, P. & Bradt, H. V. D. *Astrophys. J. Lett.* **270**, L49-L52 (1983).
14. Pedersen, H. *et al. Astrophys. J. Lett.* **270**, L43-L47 (1983).
15. Barat, C. *et al. Astrophys. J. Lett.* **286**, L5-L9 (1984).
16. Schaefer, B. E. thesis, Massachusetts Inst. Technology (1983).
17. Oort, J. H. *Bull. Astr. Inst. Neth.* **15**, 45 (1960).
18. Bahcall, J., Hut, P. & Tremaine, S. *Astrophys. J.* (in the press).
19. Ventura, J., Bonazzola, S., Hameury, J. M. & Heyvaerts, J. *Nature*, **301**, 491-493 (1983).
20. Elvis, M., Soltan, A. & Keel, W. C. *Astrophys. J.* **283**, 479-485 (1984).
21. Grindlay, J. E. *et al. Nature* **300**, 730-731 (1982).
22. Joss, P. C. & Rappaport, S. in *High-Energy Transients in Astrophysics* (ed. Woosley, S. E.) 555 (American Institute of Physics, New York, 1984).

Theory of radar imaging of internal waves

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Radar images taken over ocean areas, in particular, those obtained by the synthetic aperture radar (SAR) onboard the American Seasat satellite in 1978[1,2], sometimes show features that seem to be surface manifestations of oceanic internal waves[3-11]. Here I present a theory explaining the large radar signatures of internal waves in which the imaging is attributed to variations in the short-scale surface roughness induced by current variations associated with internal waves.

Figure 1 is an example of Seasat SAR image of coastal waters west of Portugal showing surface expressions of nonlinear internal wave trains propagating towards the coast. The internal wave groups are believed to be generated at the continental shelf break by interaction of the tidal current with underwater bottom topography. Within each group the wavelength decreases with distance from the leading crest, indicating that they represent nonlinear dispersive wave trains; their partly irregular structure is attributed to refraction effects caused by the shoaling bottom and to interactions with the current field.

It may seem surprising that internal waves can generate such large signatures in radar imagery. These signatures must orginate from the sea surface, as the penetration depth of the microwaves emitted by the radar in the sea water is only a fraction of their wavelength. The radar senses the short-scale roughness of the ocean surface by means of Bragg scattering. Thus, the radar signatures must be the result of a modulation of the short surface waves, generated by interactions with the internal waves[12]; this modulation can either be achieved by surface films (slicks) that accumulate in flow convergence zones and damp the short surface waves there[13], or by hydrodynamic interaction of these waves with the horizontal surface current associated with internal wave motion[14-17].

The first mechanism is often active in coastal waters where surface films, either of natural or anthropogenic origin, are present. These films, even when monomolecular, damp short surface waves very strongly[18-20], reducing the radar reflectivity accordingly. When slicks modulate the short-scale surface roughness, the radar image of an internal wave field consists of dark streaks (areas of reduced radar backscatter) on a uniform bright background. In most cases, however, the radar signatures of internal waves have double sign, which means that the corresponding radar image consists of bright and dark streaks associated respectively with enhanced and reduced radar reflectivity as compared with the local mean, indicative of hydrodynamic modulation. Although most investigators now hold that the latter mechanism is responsible for the imaging[7-9], the question of why these radar signatures are so large has not been answered satisfactorily. I have attempted to do so here using the weak hydrodynamic interaction theory in the relaxation time approximation, the crucial point being that another approximation is applicable compared with the case of the modulation of short by long surface waves[21-23].

Typical imaging radars operate at wavelengths λ_0 in the centimetre to decimetre range and at incidence angles θ of 20-70°, within which the radar backscattering is dominated by Bragg scattering[24-26]. In this case the normalized radar backscattering cross-section, σ, is proportional to the spectral energy density, E, of the Bragg waves, which have a wavelength of $\lambda = \lambda_0/2 \sin\theta$ and which travel towards or away from the antenna look-direction

$$\sigma = T(E(+2\boldsymbol{k}_0) + E(-2\boldsymbol{k}_0)) \quad (1)$$

where $\boldsymbol{k}_0$ denotes the projection of the radar wave vector on the horizontal plane and T a scattering coefficient which can be calculated from Bragg scattering theory and which depends on incidence angle, dielectric constant, radar wavelength and polarization (see, for example, refs 24, 26). The synthetic aperture radar on Seasat had a wavelength of $\lambda_0 = 0.235$ m (L-band) and operated at incidence angles of $\sim 23°$; the short waves responsible for the backscattering had a wavelength of 34 cm and a wave period of 0.47 s.

Equation (1) relates the modulation of the normalized radar cross-section to that of the spectral energy density $E(\pm 2\boldsymbol{k}_0)$ of the Bragg waves, which reduces the problem to that of the modulation of the Bragg waves by the surface current associated with internal waves.

Typically, internal waves visible on radar images have wavelengths greater than several hundred metres and wave periods >10 min; the space and time scales of the currents associated with these waves are much greater than those of the

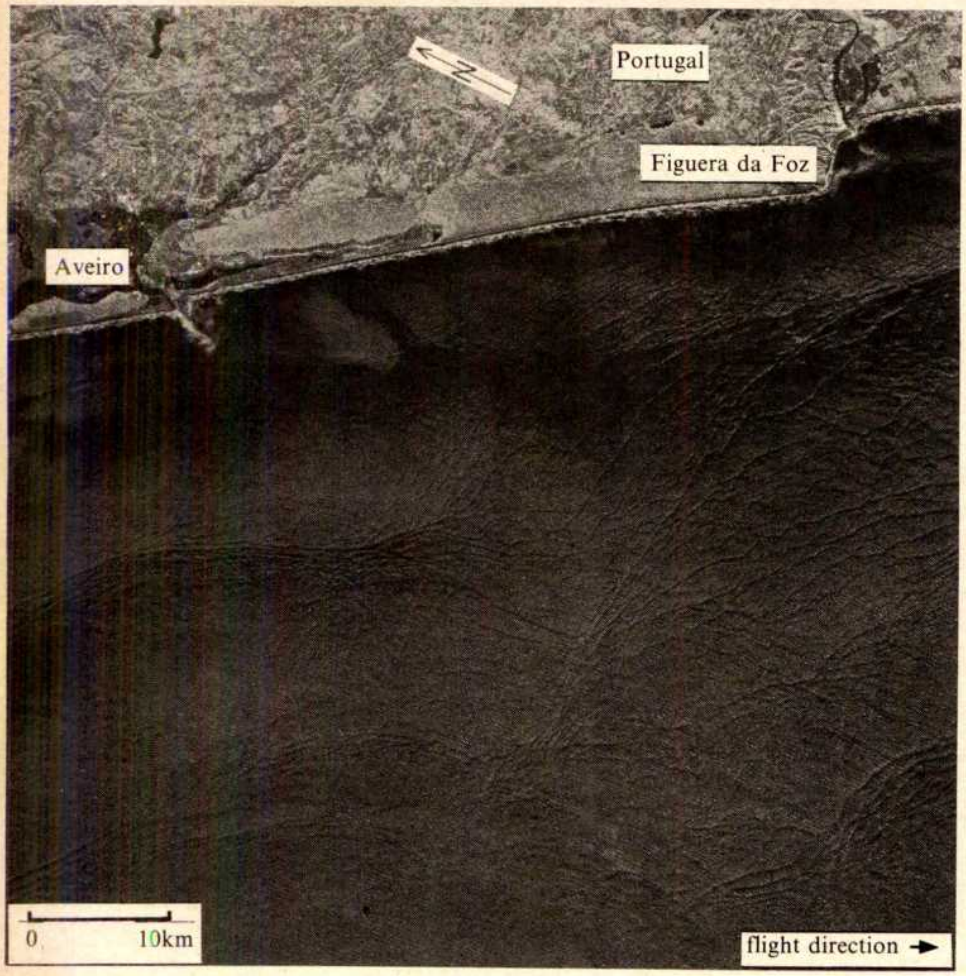


Fig. 1 Synthetic aperture radar image of Atlantic coastal waters off the Portugese coast near Figueira da Foz and Aveiro. This radar image was taken from the Seasat satellite on 20 August 1978, 21:42 UT (orbit 785). It shows surface manifestations of a large number of nonlinear internal wave trains generated by the action of the tide at the continental shelf break.

Bragg waves and therefore we can apply a Wentzel-Kramers-Brillouin-type theory to describe their interaction. In this theory the transport equation describing the variation of the spectral energy density of short waves in a slowly varying current field is the action balance or radiation balance equation[21-23,27]

$$\frac{\mathrm{d}N}{\mathrm{d}t}=\left(\frac{\partial}{\partial t}+\dot{\boldsymbol{x}}\cdot\frac{\partial}{\partial \boldsymbol{x}}+\dot{\boldsymbol{k}}\cdot\frac{\partial}{\partial \boldsymbol{k}}\right)N=S(\boldsymbol{x},\boldsymbol{k},t) \qquad (2)$$

where $N(\boldsymbol{x},\boldsymbol{k},t)=E(\boldsymbol{x},\boldsymbol{k},t)/\omega'$ is the action spectrum, $E(\boldsymbol{x},\boldsymbol{k},t)$ the wave spectrum, ω' the intrinsic frequency of the wave in a reference system which is locally at rest, $\boldsymbol{x}$ the space variable, $\boldsymbol{k}$ the wavenumber and $S(\boldsymbol{x},\boldsymbol{k},t)$ a source function. The waves propagate along trajectories in four-dimensional phase space, given by the ray equations, $\dot{\boldsymbol{x}}=\partial\omega/\partial\boldsymbol{k}$ and $\dot{\boldsymbol{k}}=-\partial\omega/\partial\boldsymbol{x}$, where $\omega(\boldsymbol{x},\boldsymbol{k},t)=\omega'(\boldsymbol{k})+\boldsymbol{k}\cdot\boldsymbol{U}(\boldsymbol{x},t)$ denotes the wave frequency in the moving medium with variable velocity $\boldsymbol{U}(\boldsymbol{x},t)$.

We assume that the variable surface current leads to only small deviations of the action density from equilibrium and therefore write the action density, N, and the surface current, $\boldsymbol{U}$, as sums of a constant equilibrium term and a time-dependent perturbation term, $N(\boldsymbol{x},\boldsymbol{k},t)=N_0(\boldsymbol{k})+\delta N(\boldsymbol{x},\boldsymbol{k},t)$ and $\boldsymbol{U}(\boldsymbol{x},t)=\boldsymbol{U}_0+\delta\boldsymbol{U}(\boldsymbol{x},t)$. Furthermore, for small perturbations we can approximate the source term S by a diagonal operator, $S(\boldsymbol{x},\boldsymbol{k},t)=-\mu\delta N(\boldsymbol{x},\boldsymbol{k},t)$, where μ is a parameter with dimension (time)$^{-1}$, called relaxation rate, and $\tau_r=\mu^{-1}$ is the relaxation time, a system constant determined by the combined effects of wind excitation, energy transfer to other waves caused by conservative resonant wave-wave interaction and energy loss through dissipative processes such as wave breaking. No direct measurements of τ_r in the open ocean exist, but theory predicts that it is of the order of 10-100 wave periods. Applied to Seasat-SAR Bragg waves, this means that τ_r should lie in the range 4.7-47 s. Inserting the above equation for N, $\boldsymbol{U}$, S,$\dot{\boldsymbol{x}}$ and $\dot{\boldsymbol{k}}$ into equation (2) and retaining only first-order terms gives

$$\left(\frac{\partial}{\partial t}+(\boldsymbol{c}_g+\boldsymbol{U}_0)\cdot\frac{\partial}{\partial \boldsymbol{x}}+\mu\right)\delta N=\boldsymbol{k}\cdot\frac{\partial \boldsymbol{U}}{\partial \boldsymbol{x}}\cdot\frac{\partial N_0}{\partial \boldsymbol{k}} \qquad (3)$$

where $\boldsymbol{c}_g=\partial\omega'/\partial\boldsymbol{k}$ is the group velocity. The time scales of the three terms on the left-hand side are the local time, τ, the advection time, τ_a, and the relaxation time, τ_r; $\tau=\Omega^{-1}$, where Ω is the radian frequency of the internal waves, and $\tau_a=|(\boldsymbol{c}_g+\boldsymbol{U}_0)\cdot\boldsymbol{K}|^{-1}$, where $\boldsymbol{K}$ denotes the wave vector of the internal wave. In general, both τ and τ_a are $\gg 1$ min; consequently, the first two terms on the left-hand side of equation (3) should be much smaller than the third, the relaxation term, which alone is retained.

Specializing equation (3) to describe the modulation of Bragg waves, defining the projection of the antenna axis on the horizontal plane as x axis, we obtain

$$\frac{\delta\sigma}{\sigma_0}=\frac{\delta N}{N_0}=\frac{\delta E}{E_0}=-(4+\gamma)\tau_r\frac{\partial U_x}{\partial x} \qquad (4)$$

Here $\delta\sigma=\sigma-\sigma_0$ denotes the deviation of the normalized radar cross-section, σ, from its mean value σ_0; γ is the ratio between the group and phase velocity of the Bragg waves ($\gamma=0.5$ for gravity waves). In deriving equation (4) we have assumed that $E\propto|\boldsymbol{k}|^{-4}$, that is, E has the form of the Phillips equilibrium spectrum[15], and that σ is related to E through equation (1). Equation (4) is our principal result here, showing that the cross-section modulation caused by internal waves is proportional to the product of the surface current gradient in the look-direction of the antenna and the relaxation time, τ_r.

An analysis of Seasat-SAR imagery of underwater sandbanks in the Southern Bight of the North Sea[27] has estimated the relaxation time of the Bragg waves; for a wind speed of 4 m s^{-1},

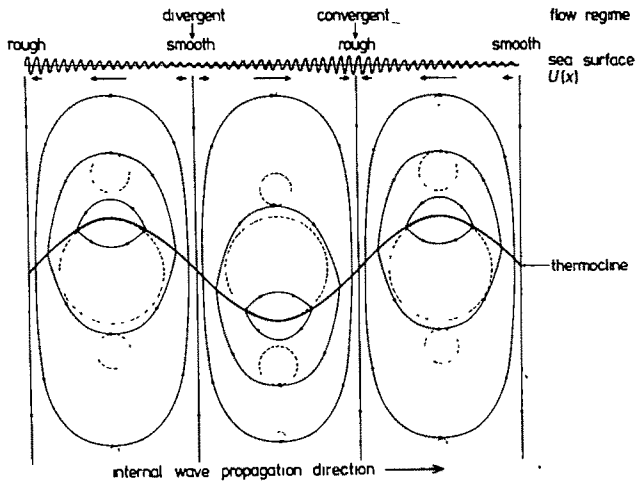


Fig. 2 Schematic plot of streamlines and particle velocities associated with a linear internal wave propagating along a sharp thermocline (adopted from ref. 28). The short-scale surface roughness is modulated by the surface current associated with the internal waves.

it is ~30–40 s, corresponding to 60–80 wave periods. This value of τ_r seems large, but is still within the predicted range.

Note that if we wanted to describe the cross-section modulation by long surface waves, equation (4) would not be applicable. In this case the corresponding equation would be

$$\frac{\delta\sigma}{\partial_0} = \frac{\delta N}{N_0} = \frac{\delta E}{E_0} = -\left(\frac{4+\gamma}{\hat{\omega}}\right)\frac{\partial U_x}{\partial x} \qquad (5)$$

where $\hat{\omega}$ denotes the radian frequency of the long surface wave and U the associated orbital velocity. Note that in equation (5) the factor in parenthesis is typically a factor of 10–20 larger than the corresponding factor in equation (4). The other factor in equation (4), the horizontal current gradient (or strain rate), $\partial U_x/\partial x$, can be calculated from internal wave theory[15].

Figure 2 shows the streamlines and water-particle orbits in a linear internal wave travelling from left to right at the boundary of two water layers of different density (thermocline). The internal waves cause almost no elevation of the surface and are associated with a spatially and temporally varying surface current field which interacts with the surface waves, giving rise to the surface-roughness modulation depicted in Fig. 2.

In the case of a nonlinear internal wave packet travelling from left to right, the vertical displacement of the thermocline and the roughness pattern have qualitatively the form shown in Fig. 3. The roughness pattern consists of pairs of adjacent very rough and smooth streaks separated by broad areas with a mean local roughness; the associated radar image therefore consists of pairs of adjacent bright and dark bands on a uniform background.

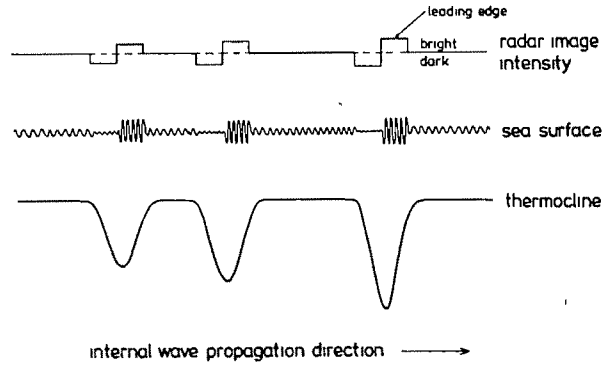


Fig. 3 Schematic plot of the thermocline displacement, the surface roughness modulation and the radar image intensity modulation associated with a nonlinear internal wave packet travelling along a shallow thermocline. The leading edge of the wave packet is associated with increased surface roughness leading to a bright band in the radar image.

The leading edge of the nonlinear internal wave train is associated with a convergence zone[29], giving rise to a bright band in the radar image.

Typical values for the strain rate of linear internal waves in coastal regions are $10^{-3}\,s^{-1}$ (ref. 7), but can be more than an order of magnitude greater for strongly nonlinear waves. If a surface current gradient of $10^{-3}\,s^{-1}$ and a relaxation time of $\tau_r = \mu^{-1} = 40$ s is inserted into equation (4), then one obtains for the modulation depth, $\delta\sigma/\sigma_0 \sim 0.2$, values commonly observed in radar imagery of internal waves. Although the surface current gradient associated with internal waves is typically an order of magnitude smaller than that associated with long surface waves, the modulation depth of internal waves can nevertheless be stronger than that of long surface waves, because the factor by which the surface current gradient is multiplied is an order of magnitude greater for internal waves (compare equations (4) and (5)).

The physical explanation for the large cross-section modulations obtained with our theory is as follows. In the case of internal waves, the straining exerted on the short-wave system by the current gradient is balanced by the relaxation of the wave system. This balance is different from the one active in the modulation of short waves by long surface waves, where the straining is balanced mainly by the temporal variation of the orbital velocity. Typically, the time scale of the relaxation (the relaxation time) is an order of magnitude greater than that of the orbital velocity, given by $\hat{\omega}^{-1}$. Thus in the case of short-wave modulation by internal waves, the straining can act sufficiently long on the short-wave system for a strong modulation to build up. Indeed the modulation is only limited by the relaxation time, which is a parameter describing how much disequilibrium the short-wave system can endure while subject to strain by a variable current.

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1 Born, G H, Dunne, J A & Lame, D B *Science* **204**, 1405–1406 (1979)
2 Jordan, R L *IEEE J Oceanic Engng* OE-5, 154–164 (1980)
3 Elachi, C & Apel, J R *Geophys Res Lett* **3**, 647–650 (1976)
4 Apel, J R in *Oceanography from Space* (ed Gower, J F R. 525–533 (Plenum, New York, 1981)
5 Vesecky, J F & Stewart, R H *J geophys Res* **87**, 3397–3430 (1982)
6. Trask, R P & Briscoe, M G. *J geophys Res* **88**, 1789–1799 (1983)
7 Alpers, W & Salusti, E *J geophys. Res* **88**, 1800–1808 (1983)
8 Hughes, B A & Gower, J F R *J geophys Res* **88**, 1809–1824 (1983)
9 Apel, J R & Gonzales, F I *J geophys Res.* **88**, 4459–4466 (1983)
10 Fu, L-L & Holt, B *J geophys Res* **89**, 2053–2060 (1984)
11 Bagg, M T. & Johnson, K I Admiralty Research Establishment, Portland (UK) Tech Note 720/84 (1984)
12 Brown, W E Jr, Elachi, C & Thompson, T W *J geophys Res* **81**, 2657–2667 (1976)
13 Lafond, E C *The Sea* Vol 1 (ed. Hill, M N) 731–751 (Interscience, New York, 1962)
14 Phillips, O M. *Phys Atmos Oceans* 9, 954–961 (1973)
15 Phillips, O M *The Dynamics of the Upper Ocean*, 199–255 (Cambridge University Press, 1977)
16 Hughes, B A *J geophys Res* **83**, 455–465 (1978)
17 Gargett, A E & Hughes, B A *J Fluid Mech.* **52**, 179–192 (1981)
18 Huehnerfuss, H, Alpers, W & Jones, W L *Radio Sci.* **13**, 979–983 (1978)
19 Huehnerfuss, H, Alpers, W, Jones, W L, Lange, P A & Richter, K. *J geophys Res* **86**, 429–438 (1981)
20 Huehnerfuss, H *et al. J geophs Res* **88**, 9817–9822 (1983)
21 Keller, W C & Wright, J W *Radio Sci* **10**, 139–147 (1975)
22 Alpers, W & Hasselmann, K. *Boundary-Layer Met* **13**, 215–230 (1978)
23 Alpers, W, Ross, D B & Rufenach, C L *J geophys Res* **86**, 6481–6498 (1981)
24 Wright, J W *IEEE Trans Antennas Propagation* AP-16, 217–223 (1968)
25 Wright, J W *Boundary-Layer Met* **13**, 87–105 (1978)
26 Valenzuela, C R. *Boundary-Layer Met* **13**, 61–85 (1978)
27 Alpers, W. & Hennings, I *J geophys Res* **89**, 10529–10546 (1984)
28 Defant, A *Physical Oceanography* Vol 2, 517–570 (Pergamon, Oxford, 1961)
29 Osborne, A R & Burch, T L *Science* **208**, 451–460 (1980)

Martian atmospheric carbon dioxide and weathering products in SNC meteorites

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SNC meteorites—four shergottites, three nakhlites and Chassigny—are postulated to have originated on Mars[1]. Their late crystallization ages (<1,300 Myr compared with 4,600 Myr for other igneous meteorites) and the presence, in shock-produced glass in EETA79001, of noble gas[2] and nitrogen[3] components resembling the martian atmosphere provide evidence for such a provenance. If this interpretation is correct then carbon dioxide, by far the most abundant constituent of the martian atmosphere[4], should also be present. The stepped combustions described here show that most of the carbon present in the samples can be ascribed to terrestrial contamination. EETA79001, however, contains 4.6 p.p.m. of an isotopically distinct component enriched in ^{13}C ($\delta^{13}C = +36‰$), whereas high-temperature carbon of inferred igneous origin in this meteorite and other SNCs has a $\delta^{13}C$ value of about −30‰. The $^{12}C/^{13}C$ ratio of the isotopically heavy component is within the error limits of Viking measurements[4] in the martian atmosphere and, thus, strengthens the case for a planetary origin. Another carbon-containing species, believed to be carbonate, has been found in Nakhla ($\delta^{13}C$ = between +12 and +24‰) and may be a product of atmospheric weathering on Mars.

Analyses of SNC meteorites were performed using a stepped combustion technique, similar to that of Swart *et al.*[5]; isotope measurements were made using a high-sensitivity static mass spectrometer capable of determining $\delta^{13}C$ values to a precision of ±3‰ or better on samples as small as 6×10^{-9} g carbon in the form of carbon dioxide (R.H.C., I.P.W., A. W. Joines and C.T.P., in preparation). $(\delta^{13}C(‰) = (R_{sam}/R_{std} - 1) \times 1{,}000$, where $R = {}^{13}C/{}^{12}C$ and $R_{std} = 0.0112372$.)

Bulk carbon contents and isotope compositions for all the meteorites obtained by summation of data from individual extraction steps are plotted in Fig. 1*a*. The total amounts of carbon present in the meteorites define three groups: (1) those recovered from the Antarctic ice cap, ignoring for the moment lithology B of EETA79001, have the lowest carbon contents (<150 p.p.m.); (2) Governador Valadares and Lafayette (>750 p.p.m. C) were found after an unknown length of time on Earth; (3) samples with intermediate carbon contents are all meteorites observed to fall and recovered soon afterwards. This pattern, which reflects the known contamination hazards[6,7] of the terrestrial environment, suggests that the carbon content of SNC meteorites is dominated by biological organic matter produced on Earth. Such a conclusion is supported by the bulk $\delta^{13}C$ data which, with the exception of Nakhla, lie in the range −20 to −30‰. Lithology B of EETA79001 was processed in a slightly different way in our laboratory and may have acquired much of its relatively large carbon content as contamination there rather than in the natural environment before collection.

The above data demonstrate the ubiquity of contamination and exemplify the difficulties involved in obtaining useful carbon isotope information from samples with a low indigenous carbon content. The problems of contamination can be overcome to some extent, however, by consideration of the carbon released during high-temperature steps of the stepped combustion. Although most terrestrial organics burn at low temperatures[5,8], it is probably not possible to choose a unique temperature for differentiating between contamination and indigenous carbon. We have chosen to consider the carbon which combusts above 600 °C. Figure 1*b* plots the summed abundance of carbon extracted above 600 °C against isotope composition. Chassigny occupies a unique position in the plot. The shergottites, with the exception of one sample of Shergotty, and the nakhlites, which are closely grouped, appear to define a two-component mixing trend, with one component an isotopically heavy species

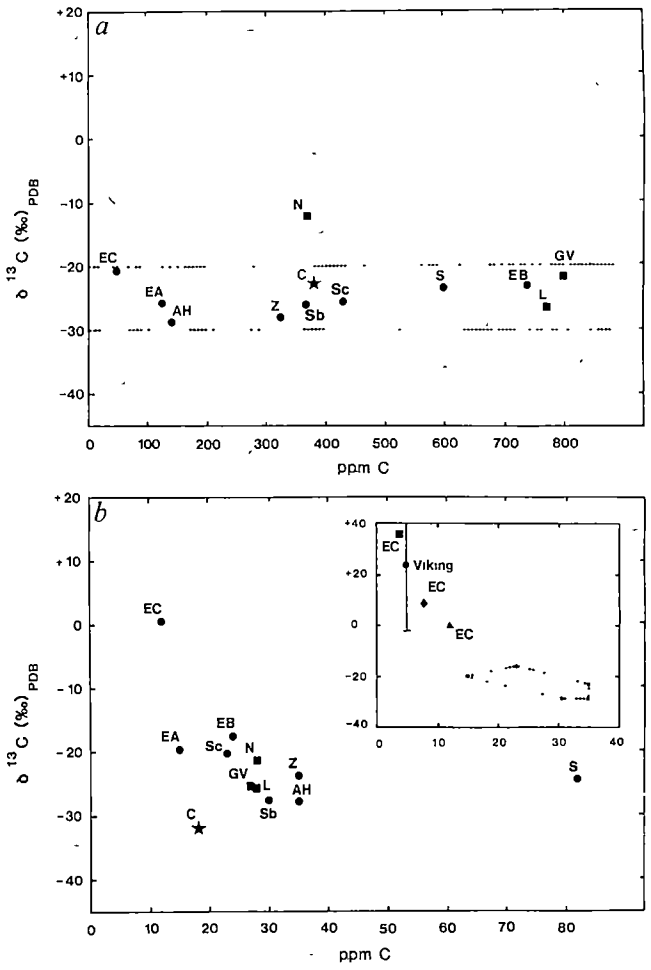


Fig. 1 *a*, Calculated bulk carbon contents and isotope compositions for samples of the shergottites (●), the nakhlites (■) and the chassignite (★). *b*, Data from >600 °C extraction steps. Inset, comparison of the SNC dataset with Viking atmospheric carbon dioxide measurements: ▲, 600–1,200 °C steps measured; ◆, 900–1,200 °C steps measured, ■, trapped atmospheric component calculated. Allan Hills 77005 (AH), Chassigny (C), Elephant Moraine 79001-lithologies A, B and C (EA, EB, EC); Governador Valadares (GV); Lafayette (L); Nakhla (N); three Shergotty samples (S, Sb, Sc) and Zagami (Z)

most easily discernible in EETA79001 lithology C. Note that about 60% of the excess carbon observed for the Shergotty sample(S) combusts at 700 °C with a $\delta^{13}C$ of −23‰, suggesting the presence of a third component.

The glass from EETA79001 is distinct from other samples studied, in that carbon released by combustion above 600 °C (Fig. 1*b*) is enriched in ^{13}C by ~20‰. Figure 2*a* shows the complete release profile for the glass; a similar profile for the host igneous material, lithology A, is shown in Fig. 2*b* for comparative purposes. The component which causes the glass (lithology C) to be enriched in ^{13}C above 600 °C is the carbon with positive $\delta^{13}C$ values released between 1,000 and 1,200 °C which is absent from the host rock.

Other authors[2,3] comparing samples of lithologies A and C of EETA79001 have noted the presence in the latter of noble gases and nitrogen with relative abundance and isotope compositions that match values measured for the martian atmosphere[4] and have accordingly argued for the existence of a trapped atmospheric component in the glass phase. The release temperatures for the proposed atmospheric components are >1,100 °C (ref. 2) and >800 °C (ref. 3) respectively. Although no isotopically heavy carbon is seen in the lithology A sample, some carbon is liberated between 900 and 1,200 °C. We take this to be indigenous carbon of igneous origin. This does not signify that we believe no terrestrial contaminants survive above

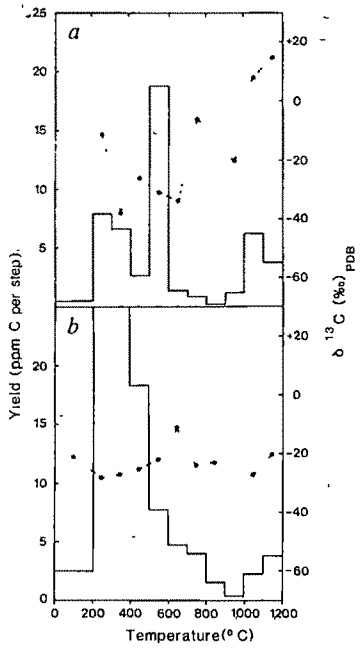


Fig. 2 Isotope composition and carbon release profile for stepped combustion of the Elephant Moraine shergottite 79001. *a*, Lithology C (glass); *b*, lithology A.

600 °C or, indeed, that indigenous components are not volatile at lower temperatures. Assuming this original carbon is completely retained during the process of shock melting, we calculate the difference between lithologies C and A (as done for nitrogen[3]) that is the trapped carbon component, to be 4.6 p.p.m. of carbon with an isotope composition of +36‰ (±10‰) ignoring any small contribution from spallogenic sources. The abundance of noble gases (and nitrogen) measured in different glass inclusions, assuming an atmospheric origin, suggest that 5 p.p.m. of trapped martian carbon dioxide ought to be present[2]. The agreement between predicted and measured abundance is extremely good. Likewise, the $^{13}C/^{12}C$ ratio corresponding to a $\delta^{13}C$ value of +36‰ is within the errors of the 'ground truth' measurements made by Viking spacecraft[4].

The inset to Fig. 1*b* shows the experimental and calculated data for EETA79001 lithology C and the atmospheric isotope composition at the predicted abundance. These points fall on the trend established from >600 °C carbon measurements and seem to support the two-component model proposed above with: (1) the heavy carbon species being atmospheric carbon dioxide; (2) the indigenous igneous carbon, by implication, having a $\delta^{13}C$ value of about −30‰; (3) shergottites and nakhlites belonging to a common provenance and having a related origin; and (4) the universally shocked shergottites being more likely to exhibit an atmospheric component trapped in glass. In relation to point (4), excess amounts of ^{40}Ar, interpreted as being from the martian atmosphere have been found in other shergottite samples[9] and we have previously pointed out the tendency for $\delta^{13}C$ values to rise at high temperatures for SNC meteorites[10]. A more convincing demonstration of the applicability of the two-component model requires separation and analyses of shock-produced glass from a variety of SNC specimens, although this may only be possible for ALHA77005.

Although not specifically related to the above discussion, other differences exist between the stepped combustion profiles of lithology A and C samples of EETA79001. A proportion of the total carbon (25%) is liberated below 500 °C for the glass; this is substantially less than for non-Antarctic meteorites and EETA79001 lithology A. The relatively high $\delta^{13}C$ value (−11‰) of the 200–300 °C extraction shows that this cannot be due entirely to biologically-produced organic contamination. Similar isotopically heavy carbon releases have been enountered with other Antarctic meteorite samples and it has been suggested that

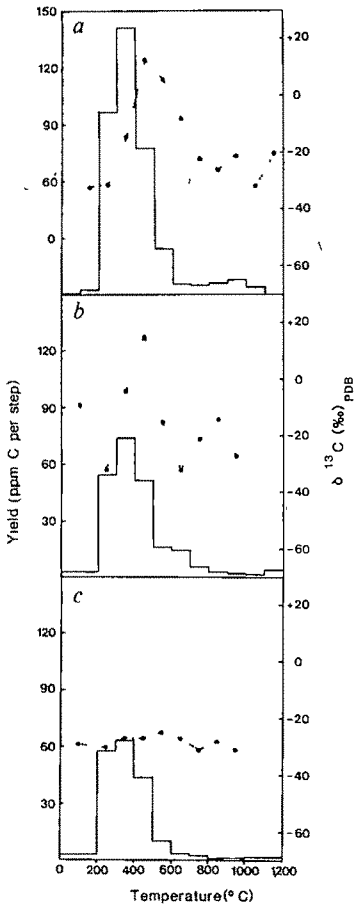


Fig. 3 Isotope composition and carbon release profiles for stepped heating: *a*, of Nakhla in oxygen; *b*, of Nakhla *in vacuo*; *c*, of the H_3PO_4 dissolution residue in oxygen.

a terrestrial weathering product may be responsible[5]. The component is presumably masked by more abundant isotopically light contamination in lithology A. Much more interesting is the large release of carbon with $\delta^{13}C = -31$‰ at 500–600 °C from lithology C but not lithology A. This component was also found for ALHA77005, another Antarctic shergottite. It may be connected with the excess of carbon in Shergotty because a larger sample of bulk Shergotty studied by Fallick *et al.*[11], also liberated substantial amounts of carbon at 600–700 °C with $\delta^{13}C = -28$‰. The source of this carbon is unknown; however, it may be related to the presence of shock-altered minerals, increased abundance in a large Shergotty specimen being consistent with a chance sampling of such material.

Nakhla has a bulk $\delta^{13}C$ of −12‰ quite distinct from all other SNC meteorites (Fig. 1*a*). This bulk isotope composition, first recognized by Fallick *et al.*[11], is dictated by the presence of a component which is volatile at <600 °C since Nakhla plots along with the other nakhlites in Fig. 1*b*. The individual steps of the stepped combusion (Fig. 3*a*) and a stepped pyrolysis (Fig. 3*b*) demonstrate that the component is, in fact, decomposed in the temperature range 300–500 °C, either with or without oxygen being present, to give carbon dioxide with a $\delta^{13}C$ rising to +15‰ in both cases. Treatment of powdered Nakhla with either hydrochloric or orthophosphoric acid removes the isotopically heavy carbon component as is shown by the stepped combustion of the residue of H_3PO_4 treatment (Fig. 3*c*). During the orthophosphoric acid reaction, carbon dioxide was collected in two steps and taken for isotope measurement. The results, given in Table 1, confirm that this gas contains carbon which is isotopically heavy, having $\delta^{13}C$ values up to +12‰. The thermal stability and chemical properties of the isotopically heavy carbon component in Nakhla are consistent with it being a carbonate present at ~0.02 wt % level. The particularly extended reaction time

Table 1 Acid dissolution of Nakhla

BM 1913,26 (43.17 mg)			BM 1911,370 (152.61 mg)		
Extraction time (h)	p.p.m. C	$\delta^{13}C$ (‰ PDB)	Extraction time (h)	p.p.m. C	$\delta^{13}C$ (‰ PDB)
18	21	+8	18	15	+6
24*	8	+12	48*	7	+46†
			24*	4	+49†
42	29	+9	90	26	+24

*Supplementary to original extraction time.

†Mass scans of these samples indicated the presence of organic species, which may have interfered with the isotope ratio measurements of the CO_2. Hence, the determined $\delta^{13}C$ values may show an apparent enrichment in ^{13}C above the true value.

recognized during H_3PO_4 treatment of a sample of a second stone from the Nakhla meteorite shower (BM1911, 360) suggests that the species is not calcite but more probably dolomite or an iron-containing carbonate. Neither of the other nakhlites show any evidence of a ^{13}C-rich component decomposing at between 300 and 500 °C, although this may be because of the release of relatively large amounts of organic material from these contaminated samples. During the stepped combustions of one sample of Shergotty and the Chassigny specimen, $\delta^{13}C$ values did increase to −12‰ and −18‰ respectively[10], but more work would be necessary to demonstrate the presence of carbonate.

A petrological investigation to confirm the presence of carbonate in Nakhla is required, but assuming that its existence is verified, the question which must be answered concerns its origin—terrestrial or martian? Terrestrial carbonates with $\delta^{13}C$ values higher than +10‰ are rare[12]. Terrestrially produced carbonates are a common feature of Antarctic weathering; all appear to have normal $\delta^{13}C$ values but such features are less conspicuous in non-Antarctic meteorites[5]. For martian carbonate to survive in Nakhla, the shock events involved in its ejection from the planet surface would presumably need to be such that temperatures did not rise to greater than 500 °C.

We have speculated that carbon dioxide in the martian atmosphere is enriched in ^{13}C relative to carbon of presumed igneous origin as present in the SNC meteorites. It may be coincidence, but recognition of enrichments of the same order for the carbonate may be circumstantial evidence for an origin by weathering in the martian environment, particularly as dolomite would be an expected product of gas-solid reaction of martian pyroxenes[13]. We note, too, that Nakhla contains iddingsite, an aqueous alteration product of olivine, which is believed to be preterrestrial in origin[14]. Unfortunately, the pyrolysis gas chromatograph-mass spectrometer experiment[15] carried out by Viking Landers, which might have been able to detect the decomposition products of carbonate and provide some isotope information was performed in such a way as to preclude such identification. It does not seem possible that the carbon dioxide trapped in EETA79001 is the relict of carbonate decomposed during shock heating, as such a route would be extremely unlikely to preserve the observed relative abundance to other atmospheric gases.

The existence of a trapped component of carbon dioxide in the correct abundance relative to noble gases and nitrogen and of an appropriate isotope composition to be of martian atmospheric origin strengthens the case that EETA79001 originated from our sister planet. The relationship between the martian atmospheric composition and data from EETA79001, other shergottites, and the nakhlites (inset in Fig. 1*b*) demonstrates that all these meteorites are related and presumably also derived from Mars. Likewise, demonstration of the existence of a carbonate mineral with a martian atmospheric composition would be direct evidence of the origin of Nakhla and its consorts from the planet.

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1 Wood, C A & Ashwal, L D *Proc Lunar planet Sci* 12B, 1359-1375 (1981)
2 Bogard, D D. & Johnson, P *Science* 221, 651-654 (1983)
3 Becker, R H & Pepin, R O *Earth planet Sci Lett* 69, 225-242 (1984)
4 Owen, T *et al J geophys Res* 82, 4635-4639 (1977)
5 Swart, P K, Grady, M M & Pillinger, C T *Meteoritics* 18, 137-154 (1983)
6 Gibson, E K Jr & Bogard D D *Meteoritics* 13, 277-289 (1978)
7 Gibson, E K Jr & Kotra, R K *Meteoritics* 17, 220-221 (1979)
8 DesMarais, D J *Proc 9th Lunar planet Sci Conf* 2451-2467 (1978)
9 Bogard, D D *Abstr 47th met Soc. Meet*, Albuquerque, J-5 (1984)
10 Carr, R H, Wright, I P & Pillinger, C T *J geophys Res* (in the press)
11 Fallick, A E *et al Abstr 14th Lunar planet Sci Conf* 183-184 (1983)
12 Deines, P *Geochim cosmochim Acta* 32, 613-625 (1968)
13 Gooding, J L *Icarus* 33, 483-513 (1978)
14 Bunch, T E & Reid, A M *Abstr 37th met Soc Meet*, Los Angeles (1974)
15 Kotra, R K, Gibson, E K Jr & Urbancic, M A *Icarus* 51, 593-605 (1982)

A high-temperature high-resolution NMR study of ^{23}Na, ^{27}Al and ^{29}Si in molten silicates

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Molecular and atomic motions over longer distances, and with much lower frequencies than interatomic vibrations, have a major role in the energetic and dynamic properties of highly structured liquids such as molten aluminosilicates. Rearrangement with increasing temperature of the polymerized anionic network (composed of linked SiO_4^{4-} and AlO_4^{5-} tetrahedra) controls viscosity and influences thermal expansion and configurational heat capacity[1,2]. Diffusive motion of network modifying cations (such as Na^+) through the structure allows electrical conductivity and probably also affects entropy. To study such motion directly and to compare liquid and glass structure, we have developed a novel high-temperature high-resolution NMR apparatus. We report here the first data on ^{23}Na, ^{27}Al, and ^{29}Si in liquids in the system $Na_2O/Al_2O_3/SiO_2$ at temperatures to about 1,300 °C. Observed changes in NMR resonant frequencies (chemical shifts) give information on the effects of composition on local structure. Line shapes and widths, and relaxation time measurements, reveal details of the dynamics and of the transition from liquid to glass.

The apparatus developed for high-temperature liquid-state NMR will be described in detail elsewhere. A sample in a 1-cm diameter boron nitride capsule is rapidly shuttled 15 cm between a furnace and a room-temperature radio frequency NMR coil, spending 10 s in the former and ~0.5 s in the latter during each cycle, during which data from a single free-induction decay (FID) is recorded. Both the probe and the furnace are mounted in a 4.2 T superconducting magnet. Sample temperatures could not be measured during actual NMR experiments, but calibration runs indicate that reported values are accurate to within about 30 °C.

Samples were prepared from reagent grade oxides and carbonates. For all ^{29}Si NMR, 95% enriched $^{29}SiO_2$ was used. Analyses of unenriched samples after the NMR runs showed that by the end of runs of 6–12 h, Na_2O contents were reduced by 5–20% of the amount present. B_2O_3 contents became as high as 4 wt%.

Measurements were made with a home-built Fourier-transform NMR spectrometer, with 90° pulse lengths of 39 μs for ^{23}Na and ^{27}Al and 45 μs for ^{29}Si. Data from 40 FIDs were averaged per spectrum for ^{23}Na, and up to 400 for ^{27}Al and ^{29}Si.

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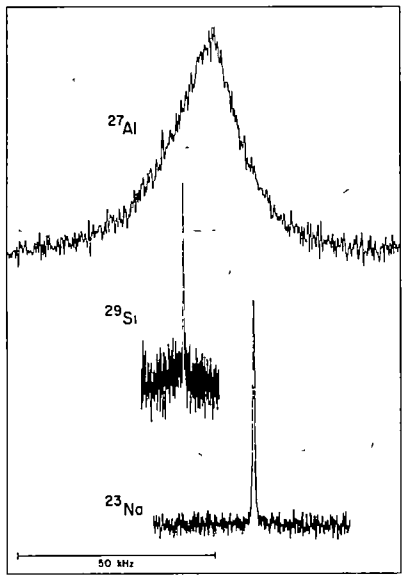


Fig. 1 Typical NMR spectra for molten silicates. Data are for $NaAlSi_2O_6$ at about 1,300 °C Relative location of the spectra is arbitrary, but all have the same frequency scale.

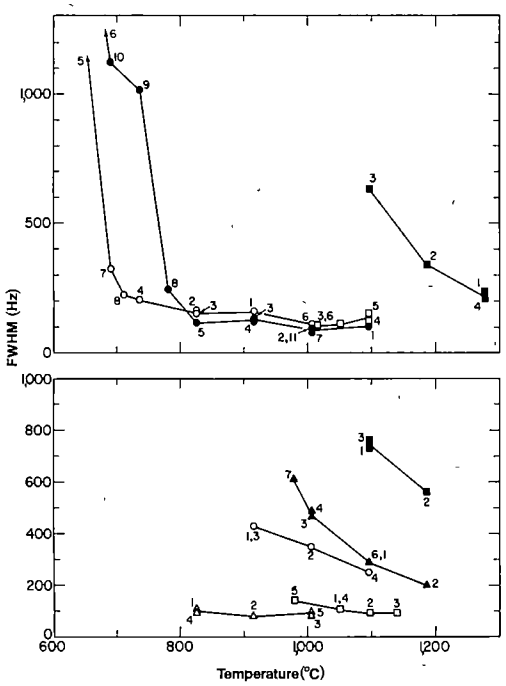


Fig. 2 Linewidths for ^{29}Si (top) and ^{23}Na (bottom) liquid spectra, as a function of temperature. Symbols indicate nominal composition. ●, $Na_2Si_4O_9$; ○, $Na_2Si_2O_5$; ■, $NaAlSi_2O_6$; □, Na_2SiO_3, ▲, 'R15' (see text), △, NaCl. Numbers show the order in which measurements were made and indicate reproducibility.

Relaxation time measurements were made by standard pulse techniques, using compensated π pulse sequences[3]. Chemical shifts are referenced to saturated aqueous NaCl (about 6.5 M), $[Al(H_2O)_6]^{3+}$, and TMS, all at room temperature

Typical spectra are shown in Fig. 1, linewidths in Fig. 2 and chemical shifts in Fig. 3. The chemical shift measurements were made at the beginning of runs when compositions were presumed to be close to nominal.

As shown in Fig. 3, ^{29}Si chemical shifts (δ) change systematically with composition, as expected from data on glassy and crystalline solids[4–7]. Liquids that are more polymerized (higher mean Q^n number, where n is the number of other AlO_4 or SiO_4 units bound to a given SiO_4 tetrahedron) have more negative δ

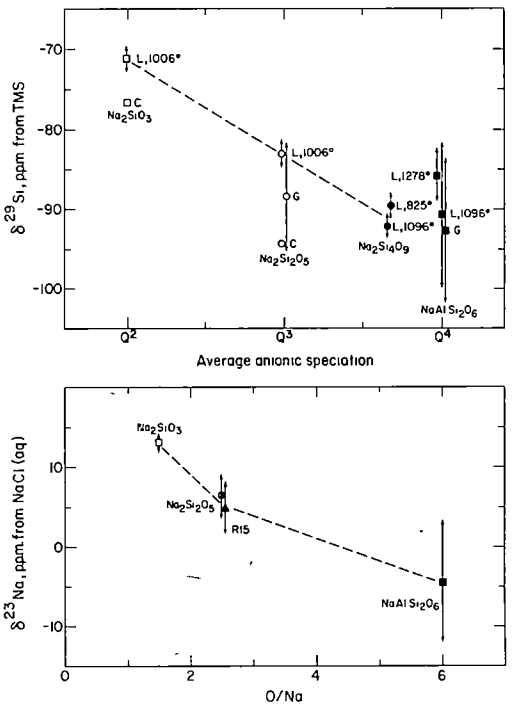


Fig. 3 Chemical shifts in silicates. Symbols as in Fig. 2, with L indicating liquid (at temperatures shown), C, crystalline solid, and G, glass (the latter two at room temperature). Arrows indicate linewidth, not errors Q^n is the average speciation of the liquid, indicating n other $(Si, Al)O_4$ tetrahedral structural groups attached to a given group.

values, showing that ^{29}Si nuclei in tetrahedral sites with greater numbers of surrounding SiO_4 tetrahedra are more chemically shielded Substitution of Al for some of the Si reduces this shielding, as shown for $NaAlSi_2O_6$ liquid and glass. Silicon chemical shifts in the liquids are systematically less negative (less shielding) than in the corresponding glasses and crystals.

Sodium chemical shifts in liquid silicates seem to have a simpler variation with composition. In all four silicate samples studied, $\delta(^{23}Na)$ is well correlated with the ratio of the number of oxygen to sodium atoms, independent of polymerization state or the Al/Si ratio. Crystalline $NaAlSi_3O_8$ (−7.7 p.p.m.)[8] falls very close to the trend for the liquids, when referenced to the same standard[9]. Our value of 6.8 p.p.m. for $\delta(^{23}Na)$ in molten NaCl agrees well with data for the solid (6.7 p.p.m.)[8].

Spectra for ^{27}Al in samples $NaAlSi_2O_6$ and R15 (Na_2O: 35, Al_2O_3: 15, SiO_2: 50 mol%) each contain only a single very broad feature (about 20 kHz wide), centred at ~29 ± 20 p.p.m. The breadth of the peaks makes it difficult to draw conclusions about Al coordination in the liquids[10].

Observed linewidths (FWHM) at the highest temperatures studied for both ^{23}Na and ^{29}Si are quite narrow (100–200 Hz, or 2–5 p.p.m.), with symmetrical lorentzian line shapes. Direct measurements of T_2 for ^{23}Na and ^{29}Si indicate that, for linewidths greater than about 200 Hz, the 'intrinsic' contribution from relaxation of spins dominates, and that, therefore, $FWHM \approx 1/(\pi T_2)$, where T_2 is the spin-spin relaxation time[11]. Smaller linewidths are dominated by instrumental effects. A few T_1 (spin-lattice) relaxation times for ^{23}Na show that $T_1 \approx T_2$ in Na_2O/SiO_2 liquids, but that $T_1 > T_2$ in the more viscous aluminosilicate liquids. Narrow high-temperature linewidths for ^{29}Si indicate that silicate species move rapidly enough to average out the effects of chemical shielding anisotropy (at least 3.5 kHz)[6], while ^{23}Na motion in the binary liquids is probably at least as fast as the NMR resonant frequency of the nucleus (or, the Larmor frequency, 47.3 MHz). For both nuclei, the FWHM increases (T_2 decreases) dramatically with decreasing temperature, as the motion that induces relaxation is 'frozen out'. For ^{29}Si, the lowest temperature at which data were

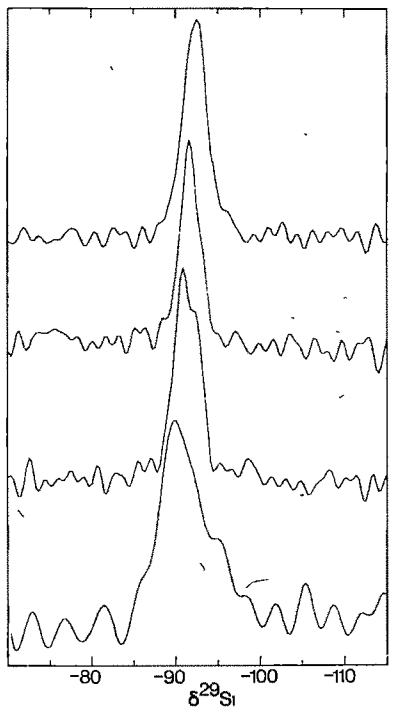


Fig. 4 ^{29}Si spectra for $Na_2Si_4O_9$ liquid at 1,096 °C (top), 1,006 °C, 915 °C, and 779 °C (bottom), normalized to the same peak height. $\delta(^{29}Si)$ is in p.p.m. Note the development of a shoulder on the central peak as the temperature is lowered.

collected was that at which the spectrum became extremely broad and 'solid-like'. In $Na_2Si_2O_5$, this was probably the result of crystallization. The $NaAlSi_2O_6$ sample, however, was recovered as a glass, indicating that the 'glass transition' with respect to molecular motion faster than 3.5 kHz was observed. Not surprisingly, more-polymerized more-viscous liquids have wider NMR lines (shorter T_2 values) at a given temperature (Fig. 2).

The very broad bands observed for ^{27}Al indicate that even though these samples were liquid, quadrupole coupling was strong enough to cause the nuclei to relax very rapidly ($T_2 \approx 20$ μs).

For Na and Si in a series of silicate liquids at high temperatures, molecular motion has a significant component at or above the kHz frequency range. At lower temperatures, the loss of this motion can be observed. The nature of this motion remains a key problem. In a discussion of liquid heat capacities, Stebbins *et al.*[2] emphasized the possibilities of rotational and translational motion of anionic molecular groups, but pointed out that mechanisms involving the breaking of strong Si–O or Al–O bonds must become important in more polymerized liquids.

Raman and magic-angle spinning NMR spectra of glasses[7,12] show that a range of silicate (Q^n) species, with a different range of chemical shifts for each value of n, make up the anionic structure of these materials. Observation of only one narrow ^{29}Si NMR line in the liquid suggests that these species chemically-exchange rapidly with respect to the typical separation in resonant frequency between the species (10–15 p.p.m. or 300–500 Hz). Only a time-averaged spectrum is observed. In aqueous silicate solutions, similar effects are seen, until temperature is lowered enough and exchange rates become slow enough to allow peak splitting to occur[13]. The development of shoulders on ^{29}Si peaks in some silicate melt spectra may suggest a similar slowing of reaction rates (Fig. 4). Structural rearrangement that includes the exchange of Q^n species involves breaking and reforming of Si–O bonds and apparently occurs even in a rather depolymerized liquid such as Na_2SiO_3. The relative contribution of this type of motion to the overall energetics of the liquid remains unknown.

The preliminary results presented here thus show that NMR spectroscopy on silicate liquids can help to identify what types of molecular motion occur, can begin to quantify the rates of such motions, and demonstrates the effects of composition. These and further data should prove useful in the understanding of both the thermodynamic properties of melts and the closely related processes of flow and diffusion.

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1 Richet, P *Geochim cosmochim Acta* **48**, 471–483 (1984)
2 Stebbins, J F, Carmichael, I S E & Moret, L K *Contr Miner Petrol.* **86**, 131–148 (1984)
3 Levitt, M H & Freeman, R *J Magn Res* **43**, 65–80 (1981)
4 Lippmaa, E, Magi, M, Samoson, A, Englehardt, G & Grimmer, A-R *J Am chem Soc.* **102**, 4889–4893 (1980)
5 Lippmaa, E, Magi, M, Samoson, A, Tarmak, M & Englehardt, G *J Am chem Soc.* **103**, 4992–4996 (1981)
6 Smith, K A, Kirkpatrick, R J, Oldfield, E & Henderson, D M *Am Miner* **68**, 1206–1215 (1983)
7 Murdoch, J B, Stebbins, J F & Carmichael, I S E *Am Miner* (in the press)
8 Kundla, E, Samoson, A & Lippmaa, E *Chem phys Lett* **83**, 229–232
9 Templeman, G J & Van Greet, A L *J Am chem Soc* **94**, 5578–5582 (1972)
10 de Jong, B H W S, Schramm, C M & Parziale, V E *Geochim cosmochim Acta* **47**, 1223–1236 (1983)
11 Farrar, T C & Becker, E D *Pulse and Fourier Transform NMR, Introduction to Theory and Methods* (Academic, New York, 1971)
12 McMillan, P *Am Miner* **69**, 622–644 (1984)
13 Englehardt, G & Hoebbel, D *JCS Chem Commun* 514–516 (1984)

Alteration of basalt glasses: implications for modelling the long-term stability of nuclear waste glasses

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The long-term stability of nuclear waste form borosilicate glasses must be predicted from extrapolations of short-term laboratory data and then verified, which is only possible by comparing the glasses with their natural analogues of geological age. Here, we suggest that basalt glasses are useful analogues for predicting the long-term behaviour of nuclear waste form borosilicate glasses. Previous workers[1–11] have considered the possibility of using natural glasses as analogues, but our study is a direct comparison of the experimentally studied corrosion products of basalt glasses, their natural long-term corrosion products and those of borosilicate glasses. Observations on the alteration processes for natural basalt glasses are combined with the experimental data on the alteration of basalt glasses and borosilicate glasses and are used to generate radioactivity release curves for nuclear waste form borosilicate glasses using the QTERM computer code[12–15]. Our data on natural glasses place significant constraints on the anticipated fraction of activity released; major differences between our two release curves do not occur until after 1,000 years.

Hydrothermal leaching experiments were completed on three glasses: two basalt glasses (KL 9 and 11) from Kilauea, Hawaii ($SiO_2 = 50$ wt%, 24 kyr old) and a simulated borosilicate nuclear waste glass, C-31-3EC-SPF-Na ($SiO_2 = 35$ wt%). We report here the results of experiments at 200 °C in saturated synthetic NaCl solution for 30 days. The basalt glasses were remelted before leaching and their chemical composition determined by electron probe analysis (EPA) (Table 1). The compositions of the surface layers and solutions were determined by optical emission spectroscopy (ICP) and the surfaces examined by scanning electron microscopy. The compositions of a basalt glass and its palagonite surface layer (deep-sea dredge sample, USNM 113715, from

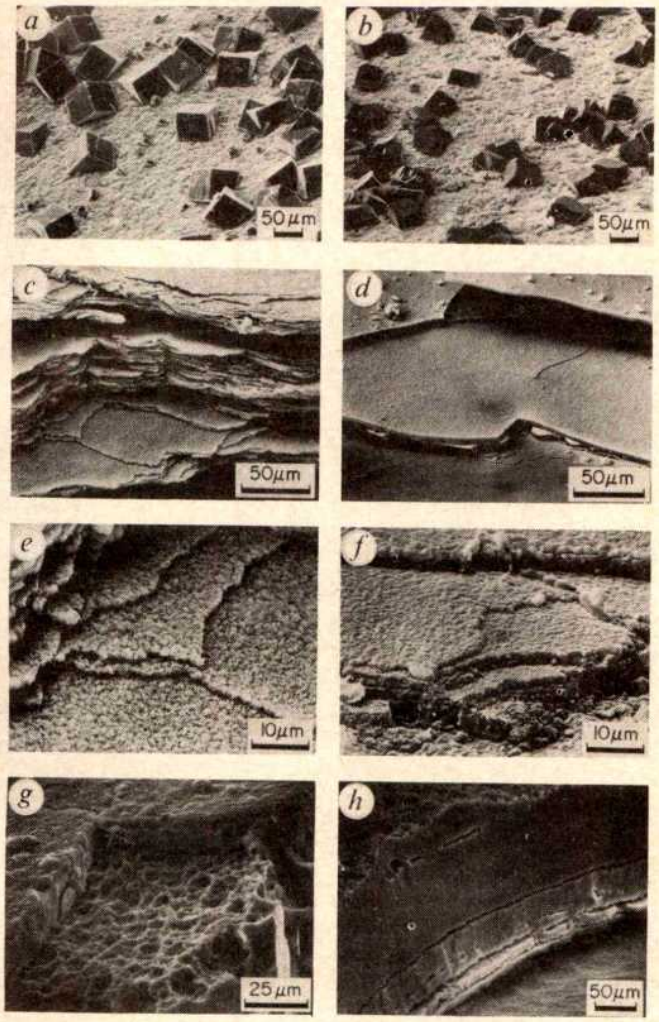


Fig. 1 Scanning electron micrographs of surface of basalt glass, KL 11, (*a*, *c*, *e*) and borosilicate nuclear waste glass (*b*, *d*, *f*) leached in saturated synthetic NaCl solution at 200 °C for 30 days. Dredge sample of basalt glass, USNM 113715, (*g*, *h*), altered in sea water at ~3 °C.

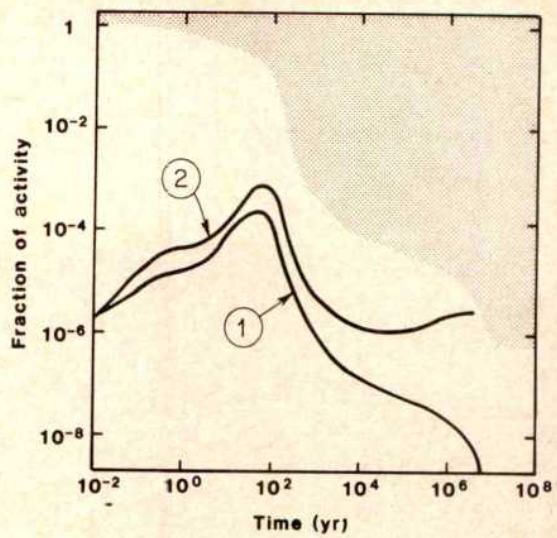


Fig. 2 Fraction of activity inventory released from the glass at any time (radioactive decay and thermal history of the waste form, with maximum temperature of 90 °C, included in the calculation of the curves). Case 1 is for parabolic time dependence; case 2 is for linear time dependence.

the Mid-Atlantic Ridge near the Kane Fracture zone, 2889–2475-m depth) are listed in Table 1 for comparison with our experimental results. This basalt glass is almost identical in composition to the normal Mid-Ocean Ridge Basalt (MORB) and the surface layer is similar to the 'average' palagonite cited by Staudigel and Hart (Table 2 of ref. 16). The dredge sample was altered by natural processes at low temperatures (~3 °C) in contact with sea water.

The most striking evidence for the similarity in the corrosion behaviour of basalt glass (KL 11) and waste form borosilicate glass (C-31-3-EC) is shown in the series of scanning electron micrographs (Fig. 1). In both cases, isometric analcime crystals were formed as surface precipitates (Fig. 1*a*, *b*). At higher magnifications, the repetitive sheet-like structures are apparent for both (Fig. 1*c*, *d*) and, at the highest magnification (Fig. 1*e*, *f*), each sheet is covered by a mat of crystal rosettes (probably clay). More importantly, a similar multiple surface layer structure (Fig. 1*e*, *f*) is common in naturally altered (~3 °C, sea water) basalt glasses (Fig. 1*g*, *h*). The same type of surface morphology on naturally altered basalt glasses has been well illustrated in previous studies[8,17,18].

The chemical similarities in the corrosion process are summarized in Tables 1 and 2. Table 1 compares the concentrations of major constituents of surface layers with the corresponding unaltered glass for basalt glasses, a borosilicate glass and a basaltic glass dredge sample. Note that in Table 1 we report only major constituents for comparative purposes (additional constituents are indicated in the footnotes). For this reason the totals of the analyses are not given, but we assume that the most important component that would bring the analyses to almost 100% is structural water. For example minerals such as vermiculite (an identified phase in surface layers) have water contents as high as 20 wt%. Although it is not possible to give totals for the analyses (because of the undetermined water content), their veracity is confirmed by mass-balance calculations (Table 2). All surface layers show depletion in SiO_2 and CaO, alumina (Al_2O_3) concentrations are approximately constant and there are increases in the concentrations of TiO_2, FeO, MnO and Na_2O in the surface layers. For the experimentally corroded glasses, the basalt glass and borosilicate glass behave similarly in terms of the amount of altered glass (as determined by weight loss and solution chemistry) and calculated depth of reaction (Table 2). For all glasses, surface layer formation is related to glass composition. The higher the ratios of Mg, Al, Fe and Ti to Si in the glass, the higher is the fraction of the surface layer relative to the total reacted glass (Table 2). The mass balance of the solution analyses and the surface analyses can be evaluated by comparing the total amount of altered glass (weight loss measurement with surface layer removed) with the sum of the altered material in the surface layer (weight measurement of surface layer) and the principal elements found in solution (SiO_2, Al_2O_3 and CaO). The data (Table 1) on the naturally altered basalt glass are used to illustrate the degree to which the experimental results of rather short-term experiments (30 days) match those for alteration processes and products of natural basalt glasses of much greater age. Our results for a naturally altered basalt glass are similar to those reported elsewhere[19–23]. There are, however, variations in the observations made among natural basalt glasses that are due to the variety of conditions, time periods and processes of alteration (see particularly Table 1 of ref. 23).

Finally, if the alteration processes and products of natural basalt glasses are satisfactory analogues of what one expects for the long-term alteration of borosilicate glasses, can this information be used to generate (or verify) models used to predict the behaviour of borosilicate glasses? Figure 2 illustrates this application by using the QTERM code (described in refs 12–15) to generate the fraction of radioactivity released for two test cases. The boundary of the shaded area in the upper portion of the figure represents the decay of the radionuclides in the canister and marks the maximum fraction of released activity if there were no borosilicate glass waste form and all activity

Table 1 Major constituents in unaltered glasses and their surface layers

Constituents (wt %)	Basalt KL 9		Basalt KL 11		Borosilicate glass C-31-3EC		Basalt‖ USNM 113715 (dredge sample)	
	Glass (EPA)	Surface (ICP)	Glass (EPA)	Surface (ICP)	Glass‡ (EPA)	Surface§ (ICP)	Glass (EPA)	Surface (EPA)
SiO_2	50.2	25.2	50.0	24.2	34.7	18.0	50.4	41.2
Al_2O_3	13.2	10.8	10.0	9.8	11.8	12.1	15.3	17.9
TiO_2	2.6	3.8	1.8	2.8	3.4	5.5	1.9	1.9
Fe_2O_3	11.1*	18.6	12.6	16.6	0.5	0.9	10.8*	14.0*
MnO	0.16	0.3	0.15	0.21	0.22	0.26	0.19	<0.05†
MgO	7.8	11.1	12.0	12.6	1.4	2.0	7.7	3.1
CaO	10.9	0.5	7.8	0.25	3.6	0.88	10.8	1.23
BaO	—	—	—	—	13.6	3.0	—	—
K_2O	0.4	−)†	0.65	−)†	—	—	0.14	2.4
Na_2O	2.2	5.4	3.6	4.1	4.8	9.2	3.0	1.6

Conditions: 200 °C, 30 days in NaCl solution, surface area/solution volume = 0.014 cm^{-1}.
* Reported as FeO.
† Below detection limit.
‡ Glass contains 22% waste oxides and 4.1% B_2O_3.
§ Data for 60 days. Layer contains 9.7% waste oxides, 5.7% ZrO_2 and 1.4% ZnO.
‖ Sample in contact with sea water at ~3 °C for geological periods of time (Mid-Atlantic Ridge).

Table 2 Summary of glass leach data

	Basalt KL 9	Basalt KL 11	Borosilicate C31-3-EC
Weight loss measurement ($g\ m^{-2}$)			
Surface layer removed	80	134	72
Surface layer	47†	92†	30†
Elements analysed in solution ($g\ m^{-2}$)			
SiO_2	24	32	16
Al_2O_3	1	1	5
CaO	8	13	15‡
Others	—	—	6§
Glass constituents retained in surface layer	Na, Al, Si as analcime, Mg*, Fe*, Ti*	Na, Al, Si as analcime, Mg*, Fe*, Ti*	Na, Al, Si as analcime, Mg*, Fe*, Ti* and some fission products
Density ($g\ cm^{-3}$)	2.837	2.862	3.265
Calculated depth of reaction (μm)	28	47	22

* In unidentified compounds.
† Includes uptake of bound H_2O and Na.
‡ Σ (CaO + BaO).
§ Σ (of oxides of Li, Zn, B, Mo, Sr).

were released immediately on contact with water. The experimental data for both cases are taken from data published by the joint research project of Japan, Switzerland and Sweden[24] where a radioactive glass with an SiO_2 content of 45 wt% was used. Experiments were performed in water at 90 °C following the MCC-1P test procedure[25]. The complete composition and experimental conditions are summarized elsewhere[24]. Data are available for up to 182 days. The essential questions are what model of corrosion is applicable, and how widely will results vary as a function of the model selected? In case 1, the time dependence is assumed to be parabolic, $t^{1/2}$ (refs 26, 27), where the most important consideration in the corrosion of the waste glass is the formation of a 'protective' layer that then becomes a diffusion barrier to further release. Beyond the 182-day database, we have continued the parabolic time dependence which has been used to describe the hydration of rhyolitic glasses[28]. In case 2, the time dependence is linear, t^1, based on the analysis of Si and B data[24], assuming an initial first-order dissolution reaction for the glass with silica approaching saturation. Between 28 and 182 days, glass corrosion proceeded at a low but constant rate as indicated by boron release. Beyond the 182-day database, we have continued the linear time dependence, as required in describing the formation of palagonite rinds on basalt glasses[29]. The latter case is the most conservative (maximum) estimate of the released activity. The curves for cases 1 and 2 are only illustrative (as the temperature dependence of palagonitization of natural basalt glass and the alteration of waste glasses is still under investigation), but they do provide us with important examples of time-limited experimental data combined with information derived from the alteration of natural glasses over long periods of time.

Three important points may be made. (1) Depending on the model used to extrapolate the long-term release of radionuclides, there are wide variations in the fraction of released activity (for example, at 10 kyr, an order of magnitude). (2) There is little difference between the two cases in the fraction of activity released during the first 100 yr, but afterwards there is an increasing difference. At 1 Myr, for case 2, there is little difference between the fraction of activity released and the total fraction of remaining activity due to radioactive decay (the intersection of the release curve with the shaded area), whereas at 1 Myr, for case 1, the waste form is still able to reduce the fraction of activity released by two orders of magnitude. (3) Data on naturally altered basalt glasses (that is, time dependence of corrosion, role of layer formation in release of elements and identification of stable phases in the precipitates) can be used in presently available release codes (QTERM). The use of the appropriate natural analogue is of immense value in designing and verifying release codes over long periods of time.

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1. Ewing, R. C. *Scientific Basis for Nuclear Waste Management* Vol. 1 (ed. McCarthy, G.) 57-68 (Plenum, New York, 1979).
2. Ewing, R. C. & Haaker, R. F. *Naturally Occurring Glasses: Analogues for Radioactive Waste Forms* (Battelle Rep. PNL-2776/UC-70, 1979).
3. Adams, P. B. *Int. Symp. Ceramics in Nuclear Waste Management* (eds Chikalla, T. D. & Mendel, J. E.) 233-237 (American Ceramic Society, 1979).
4. Malow, G. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management* Vol. 3 (ed. Moore, J. G.) 315-322 (Plenum, New York, 1981).
5. Dickin, A. P. *Nature* **294**, 342-345 (1981).
6. Melling, P. J., Vemparti, C. S., Allnatt, A. R. & Jacobs, P. W. M. *Physics and Chemistry of Glasses* **22**, 49-54 (1981).
7. Bates, J. K., Jardine, L. J. & Steindler, M. J. *Science* **218**, 51-53 (1982).
8. Allen, C. C. *Scientific Basis for Nuclear Waste Management-V* (ed. Lutze, W.) 37-44 (Elsevier, New York 1982).
9. Barkatt, A. *et al. Geochim. cosmochim. Acta* **48**, 361-372 (1984).
10. Plodinec, M. J., Jantzen, C. M. & Wicks, G. G. *2nd Int. Symp. Ceramics in Nuclear Waste Management* (eds Wicks, G. G. & Ross, W.) 491-495 (American Ceramic Society, 1984).
11. Malow, G., Lutze, W. and Ewing, R. C. *J. non-crystalline Solids* **67**, 305-321 (1984).
12. Altenhein, F. K., Lutze, W. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management-V* (ed. Lutze, W.) 45-56 (Elsevier, New York, 1982).
13. Altenhein, F. K., Lutze, W. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management-VI* (ed. Brookins, D.) 269-280 (Elsevier, New York, 1983).
14. Altenhein, F. K., Lutze, W. & Ewing, R. C. *2nd Int. Symp. Ceramics in Nuclear Waste Management.* (eds Wicks, G. G. & Ross, W.) 636-644 (American Ceramic Society, 1984).
15. Freude, E., Grambow, B., Lutze, W., Rabe, H. & Ewing, R. C. *Scientific Basis for Nuclear Waste Management-VIII* (eds Jantzen, C. M., Stone, J. A. & Ewing, R. C.) (Elsevier, New York, in the press).
16. Staudigel, H. & Hart, S. R. *Geochim. cosmochim. Acta* **47**, 337-350 (1983).
17. Allen, C. C., Gooding, J. L., Jercinovic, M. J. & Keil, K. *Icarus* **45**, 347-369 (1981).
18. Jercinovic, M. J. thesis, Univ. New Mexico (1984).
19. Ailin-Pyzik, I. B. & Sommer, S. E. *J. geophys. Res.* **86**, 9503-9510 (1981).
20. Melson, W. G. *Trans. Am. geophys. Un.* **54**, 1011-1014 (1973).
21. Melson, W. G. & Thompson, G. *Bull. geol. Soc. Am.* **84**, 703-716 (1973).
22. Schmincke, H.-U. *Init. Rep. DSDP* **LXV**, 477-483 (1983).
23. Honnorez, J. *The Sea* (ed. Emiliani, C.) 525-587 (Wiley, New York, 1981).
24. Hermansson, H.-P., Bjorner, I.-K., Christensen, H. & Yokoyama, H. *Studsvik Rep.* NW-84/653 (1984).
25. MCC-1P, Static Leach Test Method *Nuclear Waste Materials Handbk* (US Department of Energy, DOE/TIC-11400, 1981).
26. Wicks, G. G. & Wallace, R. M. (Savannah River Laboratory, DOE Rep. DP-82-18, 1982).
27. Wicks, G. G. & Wallace, R. M. *Scientific Basis for Nuclear Waste Management-VI* (ed. Brookins, D.) 23-28 (Elsevier, New York, 1983).
28. Friedman, I., Smith, R. L. & Long, W. D. *Bull. geol. Soc. Am.* **77**, 323-328 (1966).
29. Morgenstein, M. & Riley, T. J. *Asian Perspectives* **17**, 145-159 (1974).

Flow law for ice in polar ice sheets

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Theories of glacier flow are based commonly on the assumption that ice is not a newtonian fluid, but has a non-linear stress-dependent viscosity[1-5]. Here we re-examine the spreading of Antarctic ice shelves and suggest that the data cannot define a unique flow law. Tilt measurements in four boreholes in both the Arctic and Antarctic seem to show that a linear flow law $\dot{\varepsilon} = A_1\tau$ (where $A_1 \sim 10^{-15}\,s^{-1}\,Pa^{-1}$ at $-28\,^{\circ}C$), where $\dot{\varepsilon}$ is the effective strain rate and τ the effective shear stress, may be just as appropriate for describing the flow of polar ice sheets.

Conventional flow laws for ice are written as[3] $\dot{\varepsilon} = A_n\tau^n$. A_n is often assumed to be modelled adequately by an Arrhenius temperature dependence, $A_n = A_0 \exp(-Q/RT)$, where Q is the activation energy, R the gas constant and T the absolute temperature. To allow for a possible pressure dependence, T is referred to the *in situ* melting point. A_0 is assumed to include the effects of parameters that describe variations in flow, such as grain size, ice fabric and impurity content. According to laboratory experiments, the exponent n can range from 1 at low stresses (below $\sim 10^4$ Pa) to 4 or even higher at stresses $> 10^5$ Pa (ref. 6), in agreement with field data for borehole closure[7], where $n = 3$ was found for $\tau = 2 \times 10^5$-10^6 Pa, although these latter data may include a large component of transient creep.

The stresses that are important for ice-sheet flow, however, lie in the range 10^4-10^5 Pa. Data from spreading of ice shelves have been interpreted[8] as showing that, in this stress range, n is ~3, which is now the generally accepted value, although Whillans[9], using an empirical formulation of the flow law derived from tilting of boreholes, found the stress exponent varied between 1 and 2. Low values of n at low stresses have been explained as the effects of transient creep. Paterson[3,10], reviewing laboratory and field data, has shown that there is a large spread in experimental results, such that under similar conditions strain rates may vary by more than an order of magnitude; but by selecting measurements thought to be most reliable, he could recommend[3] values of $n = 3$, $Q = 60$ kJ mol^{-1} and $A_3 = 5.2 \times 10^{-25}\,s^{-1}\,Pa^{-3}$ at $-10\,^{\circ}C$. Further analysis of borehole data has revealed that Holocene ice in both Greenland and Antarctica was ≤ 20 times softer than expected from ice-shelf spreading data[10]. We have attempted to reduce this discrepancy by re-examining ice shelf and borehole data from several sites, without assuming *a priori* that $n = 3$. We have not attempted to determine a flow law in the rheological sense, but only to find those values for the above parameters that give the best agreement with the field results.

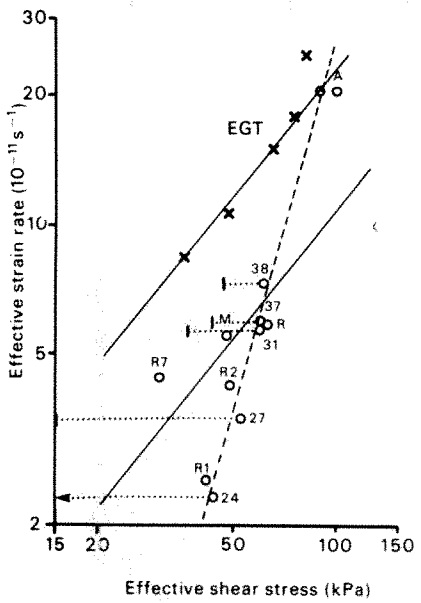


Fig. 1 Plot of effective strain rate against effective stress for ice-shelf sites[8] (A = Amery; M = Maudheim; R = Ross; R1, R2, R7, 24, 27, 31, 37, 38 = Brunt), with an $n = 3$ line (dashed). Error bars show the possible influence of the restraining force from MacDonald Ice Rumples. Solid line is an $n = 1$ line that falls within the error bars. The uppermost line and crosses are for Erebus Glacier Tongue[11].

Ice-shelf data (strain rates and ice thickness) were used by Thomas[8] for sites selected to conform as closely as possible to the ideal of an unbounded ice shelf. Being unable to measure the stress field directly, he calculated the effective shear stress averaged over depth by assuming that there was no restraint by lateral shear at side walls and ice rises and that the only longitudinal restraint was by sea water pressure acting along the direction of the maximum principal strain rate. Figure 1 shows the effective strain rates plotted against effective shear stresses. Thomas[8] stated that a flow law with $n = 3$ fitted the data. Not only were all the sites constrained laterally to a greater or lesser extent, thus giving lateral shear restraint, but also the Brunt Ice Shelf sites, which are the main constraint on the $n = 3$ line, were influenced by MacDonald Ice Rumples. Using his derived flow law, Thomas[8] calculated a significant restraining force exerted by MacDonald Ice Rumples on Brunt Ice Shelf. Although for more distant sites the direction of the force is almost perpendicular to the direction of the maximum principal strain rate and may therefore be considered to have been accounted for by the lateral strain rate, there could still be a lateral shear restraint. Because of this uncertainty in describing the stress field, we show in Fig. 1, for the relevant Brunt Ice Shelf site, error bars on the effective shear stress whose magnitudes are derived from Thomas's plot of restraining force against distance from MacDonald Ice Rumples[8]. The linear flow law line $\dot{\varepsilon} = A_1\tau$, which passes through the data from Maudheim and Ross Ice Shelf, lies well within these error bars.

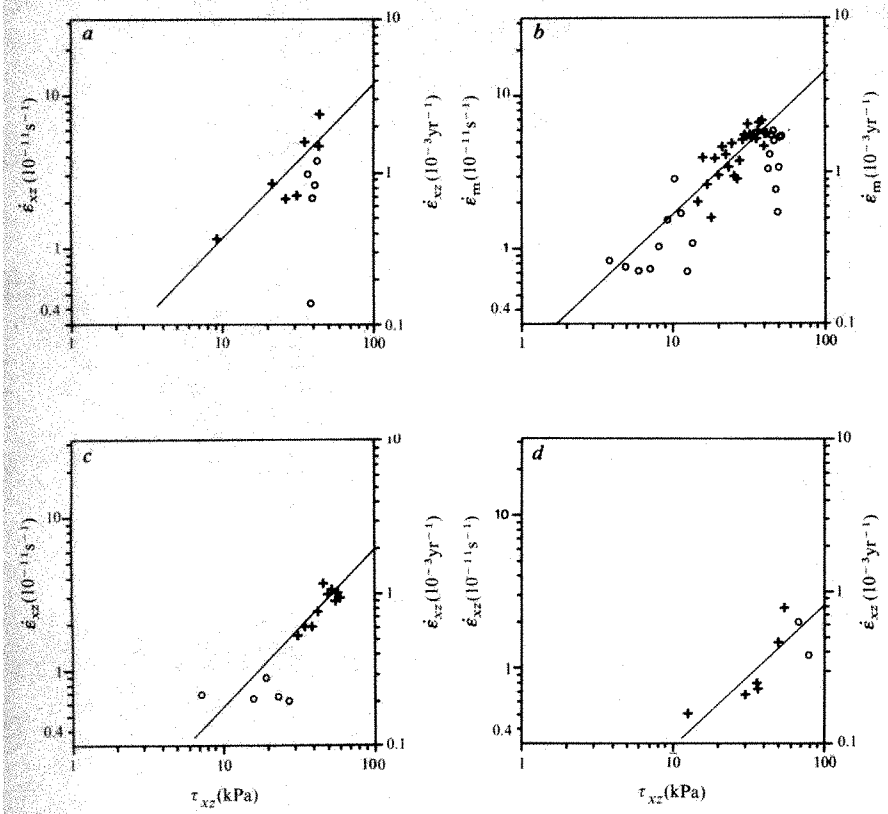


Fig. 2 Logarithmic plots of $\dot{\varepsilon}_{xz}$ against τ_{xz} from the tilting rate of boreholes. *a*, Camp Century; *b*, Byrd Station; *c*, Devon Island; *d*, Law Dome. The Byrd Station and Law Dome data are corrected as described in the text. Data used in the regression are marked as crosses, the rest as circles and the line of best fit is shown.

Erebus Glacier Tongue (EGT) is perhaps a closer approximation to an unbounded ice shelf. Holdsworth[11] interpreted its spreading by assuming that $n = 3$, and he was forced to postulate large variations in the value of A_3. We have plotted these data on Fig. 1. With the exception of the point of highest stress, which is only 2 km from the hinge line and where the velocity field (and therefore strain rate) is uncertain, the points fall closely on a line with $n = 1$. At the mean estimated temperature of −13 °C, the value of A_1 is 2.6×10^{-15} s^{-1} Pa^{-1}. In summary, it seems that, because of the uncertainty in the stress fields, the ice-shelf data cannot be used to determine a unique flow law, although the ice shelf that is closest to being unbounded is that which most strongly suggests a linear law.

Tilt measurements have been made over several years to near bedrock at Camp Century[10,12] in Greenland and Devon Island[13] in Arctic Canada, and for about the top 70% of the ice thickness at Byrd Station[10,12] and Law Dome[14] in Antarctica. Paterson[10] has plotted a restricted data set for Camp Century and Byrd Station on a logarithmic plot of $\dot{\varepsilon}_{xz}$ against τ_{xz} where the axes x, y, z define a rectangular coordinate system, x is the direction of flow and z is vertically downwards. He obtained a reasonable fit to curves computed using $n = 3$, but only when using a value of A_3 15–20 times that previously recommended. But for these few scattered data an equally good or better fit is obtained if curves are computed using $n = 1$. In Fig. 2*a*–*d* we have plotted for the four sites all the data, referred to a temperature of −28 °C, using an activation energy of 60 kJ mol^{-1}.

For Camp Century and Devon Island, we have simply plotted the temperature-compensated $\dot{\varepsilon}_{xz}$ against τ_{xz}, but for Byrd Station, flow in the upper part of the hole can be perpendicular to the surface gradient and in Fig. 2*b* we used, following Paterson[10], a resolved strain rate $\dot{\varepsilon}_m = (\dot{\varepsilon}_{xz}^2 + \dot{\varepsilon}_{yz}^2)^{1/2}$. The reason for the spiralling flow at Byrd is unknown, but it seems reasonable to follow earlier investigators in assuming that the flow, in whatever direction, is driven ultimately by shear stresses related to the surface slope. For Law Dome, laboratory measurements have been made[14] of the relative softness of ice from different depths. Because the relevant experiment used strain rates determined at a given stress and temperature, it does not rely on the assumption of any flow law. The variations in softness were related to ice fabric and were reported as enhancement factors (f) between 1 and 4. In Fig. 2*d*, we have plotted $\dot{\varepsilon}_{xz}/f$ compensated to −28 °C, to remove, where possible, variations in flow resulting from factors other than stress dependence; the two highest stress points were obtained from an extrapolation of strain rates to bedrock, assuming zero basal velocity[14].

As we have allowed for temperature change, we might expect the data in Fig. 2 to fall on a straight line with gradient n, the flow law exponent. Quasi-linearity (that is, gradient = 1 on this plot), however, would be observed for a power law in regions where longitudinal stresses dominate. Therefore, in our regression analysis to find the best value of n, we have discarded all data above the greatest depth (for each site) at which the resolved longitudinal strain rate (taken as the measured surface value, Table 1) is greater than $\dot{\varepsilon}_{xz}$ (or $\dot{\varepsilon}_m$). Following Paterson[10], we have included neither the data for Wisconsin ice, as its flow could be affected by other factors, nor the two extrapolated points at Law Dome. Regressions on the remaining data (shown by crosses in Fig. 2) gave n values of: 1.04 for Camp Century, 0.93 for Byrd Station, 1.03 for Devon Island and 0.8 for Law

Table 1 Measured surface longitudinal strain rates

		Camp Century[10]	Byrd Station[10]	Devon Island[16]	Law Dome[17]
Strain rates (10^{-4} yr^{-1})	$\dot{\varepsilon}_{xx}$	2.6	4.4	6.7	2.3
	$\dot{\varepsilon}_{yy}$	0	1.4	0.2	5.7

Table 2 Values and ratios of A_1 for ice shelves and boreholes (at −28 °C)

	Erebus Glacier Tongue	Camp Century	Byrd Station	Devon Island	Law Dome
A_1 (s^{-1} Pa^{-1})	4.8×10^{-16}	1.5×10^{-15}	1.4×10^{-15}	5.4×10^{-16}	2.1×10^{-16}
Ratio:	1	3	3	1	0.4

Law Dome has been corrected for laboratory measured enhancement factors; the value and ratio should be doubled to be compared with other sites.

Dome. Equivalent analyses using an activation energy of 40 kJ mol^{-1} give similar results, with n between 0.95 and 1.2 for the four sites.

Our results must be qualified because the difficulty in making accurate inclination measurements means that, generally, tilt data are of fairly poor quality, especially in the upper part of the borehole. Regressions through even the deeper data, however, would still give $n \ll 3$. This, together with the fact that all four boreholes suggest the same result, indicates that random errors in the poor quality data have not influenced the result unduly. In the upper layers of an ice sheet there may also be a component of transient creep resulting from the steadily increasing shear stress as the ice moves downwards, but this will also be a feature of the flow of the ice sheet as a whole, and is therefore a factor which a realistic analysis should not exclude.

Our second method of analysis allows explicitly for the effect of longitudinal stresses. For each datapoint we have calculated the value of the flow-law constant A_n normalized to a given temperature (−28 °C) by

$$A_n = \left(\frac{\dot{\epsilon}_{xz}}{\rho g \alpha z}\right)^n \exp\left[\frac{Q_n}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)\right] \Big/ E^{n-1} \qquad (1)$$

where n is the stress exponent (either 1 or 3), $\dot{\epsilon}_{xz}$ the vertical-shear strain rate, ρ the density, g the acceleration due to gravity, z the depth, α the surface slope, Q_n the activation energy, $T_0 = -28$ °C and E^2 the second invariant of the strain-rate tensor. Local variability in the values of A results from unmodelled factors such as grain size, fabric or impurities. The general trend for all sites, however, is of a constant value of A_1 for a linear law, with the average A_1 in the top half of each hole very close to that found in the bottom half. Alternatively, for a power law ($n = 3$), A_3 shows a consistent decrease with depth over several orders of magnitude. If a power law had been most appropriate at all depths, the trend should have been for a constant value of A_3 and an increasing value of A_1 with depth.

If we assume a linear law applies, then values of A_1 averaged over depth are given by the gradient of plots of $\dot{\epsilon}_{xz}$ against τ_{xz} on linear axes. Using the same data as before gives the values in Table 2; a value for Erebus Glacier Tongue is also given, corrected to a temperature of −28 °C using an activation energy of 60 kJ mol^{-1}. The values agree to within a factor of four, compared with the factor of 15–20 needed by Paterson[10] to reconcile borehole and ice-shelf data using a power law with $n = 3$.

Another way of comparing flow laws is to consider horizontal velocity profiles with depth. These are obtained by integrating the measured borehole shear rates downwards from the surface to the lowest point at which they were measured. If the surface velocity is known, then an upper bound can be placed on the resulting deformation in the lowest unmeasured layers. At Byrd Station, extrapolating the average value of A_1 to the bed and using a linear law suggests a sliding velocity of 1.4 m yr^{-1}, which is reasonable because of the water at the bottom of the borehole[15]. Using $n = 3$ and the average value of A_3 calculated for the last 250 m of measured tilting gives a negative velocity at the bed. There must be another reduction in A_3 (by an order of magnitude) for the basal velocity to be zero. At Law Dome, to avoid negative basal velocities calculated using $n = 3$, Russell-Head and Budd[14] suggested that shear stresses reached a maximum at two-thirds depth and then decreased towards the bed. This postulate is unnecessary with $n = 1$, where values of A_1 can be extrapolated to the bed to give zero velocity there.

For all four boreholes, a linear law gives overall a more coherent explanation of the data and one which agrees better with ice-shelf data. If $n = 3$ were the correct rheological exponent, then such large variations in A_3 would be required (with depth and site) that the flow law would cease to have any predictive value. We conclude that on evidence available at present it is not necessary to use a non-linear flow law to describe the flow and deformation of polar ice sheets.

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1. Nye, J. F. *Proc. R. Soc.* **A219**, 477–489 (1953).
2. Glen, J. W. *Proc. R. Soc.* **A228**, 519–538 (1955).
3. Paterson, W. S. B. *The Physics of Glaciers* 2nd edn (Pergamon, Oxford, 1981).
4. Hooke, R. le B. *Rev. Geophys. space Phys.* **19**, 664–672 (1981).
5. Weertman, J. *A. Rev. Earth planet. Sci.* **11**, 215–240 (1983).
6. Lile, R. C. *ANARE Sci. Rep.* No. 132 (1984).
7. Paterson, W. S. B. *Rev. Geophys. space Phys.* **15**, 47–55 (1977).
8. Thomas, R. H. *J. Glaciol.* **12**, 55–70 (1973).
9. Whillans, I. M. *J. geophys. Res.* **86**, 4272–4282 (1981).
10. Paterson, W. S. B. *Cold Regions Science and Technology* **8**, 165–179 (1983).
11. Holdsworth, G. *Ann. Glaciol.* **3**, 131–137 (1982).
12. Robin, G. de Q. in *The Climatic Record in Polar Ice Sheets* (ed. Robin, G. de Q.) 98–118 (Cambridge University Press, 1983).
13. Paterson, W. S. B. & Waddington, E. D. *Rev. Geophys. space Phys.* **22**, 123–130 (1984).
14. Russell-Head, D. S. & Budd, W. F. *J. Glaciol.* **24**, 117–130 (1979).
15. Ueda, H. T. & Garfield, D. E. *Int. Ass. Sci. Hydrol. Publ.* **86**, 53–62 (1970).
16. Paterson, W. S. B. *J. Glaciol.* **17**, 3–12 (1976).
17. MacLaren, W. A. *ANARE Sci. Rep. Ser. A(IV)* No. 103 (1968).

Sulphate sequestered in the sulphate-binding protein of *Salmonella typhimurium* is bound solely by hydrogen bonds

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An important question in understanding substrate binding by proteins is how charged groups are stabilized in the absence of their solvation shell. We have addressed this question here by solving the structure of the sulphate-binding protein of *Salmonella typhimurium* with bound substrate at 2.0 Å resolution. The results are remarkable in that the charged oxygen atoms of the sulphate molecule, which is buried and completely inaccessible to the solvent, are not stabilized by the formation of salt-bridges but by hydrogen bonds donated by specific residues of the protein. These hydrogen bonds are in turn coupled via peptide units to several resonating hydrogen bonding systems. These findings may be of general significance for the role of electrostatic interactions in protein structure and function.

In addition to the sulphate-binding protein, we have recently refined the molecular structures of three other binding proteins specific for L-arabinose[1], D-galactose and Leu, Ile or Val (N. K. Vyas, M. N. Vyas, J. S. Sack and F.A.Q., unpublished data; see also refs 2, 3). These periplasmic proteins, with dissociation constants of ~10^{-6} M, belong to the family of proteins which act as initial receptors for active transport systems and chemotaxis in bacteria[4]. All four structures are ellipsoidal and composed of two similar globular domains connected by a flexible hinge.

The tertiary structure of the sulphate-binding protein at *p*H 4.3, which consists of 2,570 non-hydrogen atoms from 308 residues, 1 sulphate and 143 ordered water molecules, was refined by the method of Hendrickson and Konnert[5] to an *R*-factor of 0.14 for the 16,959 reflections from 8 to 2.0 Å. The refined structure differs from ideal bond lengths and angle distances by overall r.m.s. deviations of 0.015 Å and 0.038 Å, respectively. A more detailed description of the structure determination and protein conformation will be presented elsewhere.

Figure 1 shows the location of the binding site and Fig. 2 and Table 1 illustrate the precise details of sulphate binding. The sulphate is buried in the cleft ~7 Å below the protein surface and becomes completely inaccessible to the solvent; none of the 73-Å^2 accessible surface area[6] of the free sulphate is exposed. There are no positively charged residues, cations or water molecules within van der Waals' distance of the sulphate, thus precluding the formation of salt-bridges to neutralize the charged sulphate. Instead, all of the sulphate oxygens are the recipients of hydrogen bonds from seven residues distributed

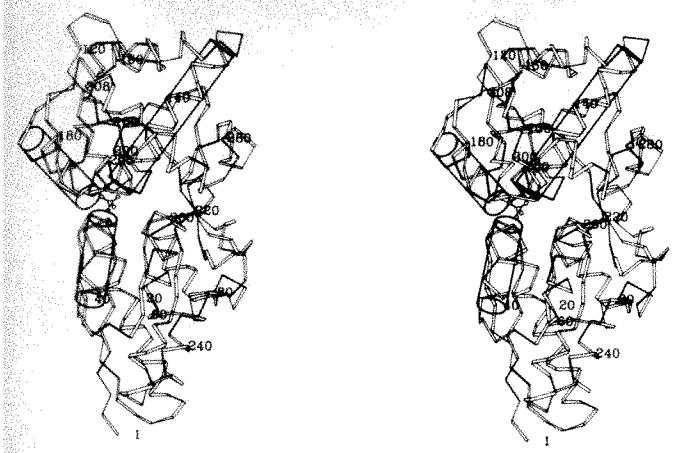


Fig. 1 Stereo view of the Cα backbone trace of the sulphate-binding protein, with the sulphate bound in the cleft between the two domains. The sulphate is shown in a ball-and-stick model and the three helices (44–54, 131–146, 172–181) close to the sulphate are further enclosed in cylinders. The residues hydrogen-bonded to the sulphate originate from both domains: the N-terminal domain supplies Asp 11 and Ser 45 and the C-terminal domain provides Ser 130–Gly 131–Gly 132–Ala 133, Ala 173 and Trp 192. Because of four likely changes to the published sequence of 310 residues[15] as a result of the present refinement, the sequence numbering scheme used in this study is based on the refined structure. However, as these changes (none in the binding site) consisted of one insertion, one deletion and two amino-acid substitutions, the total number of amino acids is unaltered. The last two residues are highly disordered. (Drawing produced by ARPLOT[16].)

Table 1 Hydrogen bonds between sulphate and binding protein

Protein atoms, donor (X)	Sulphate atoms, acceptor (Y)	Distance (Å) X···Y	Angle (°) X–H···Y
Ser 130 OG	O-1	2.76	167
Ala 173 N	O-1	2.70	151
Ser 45 N	O-2	2.84	169
Gly 131 N	O-2	2.83	170
Gly 132 N	O-3	2.77	149
Trp 192 NE1	O-3	2.82	157
Asp 11 N	O-4	2.67	152
	Overall mean:	2.76 (0.07)	159 (9)

Hydrogen bonds were determined on the basis of angle and distance criteria and hydrogen atoms were located on the basis of standard geometry (see ref. 1).

in both domains. These H-bonding residues are also buried. The sequence Ser 130-Gly 131-Gly 132-Ala 133, which forms part of a loop and the first turn of a helix, has a key role in binding, as the first three residues provide a complementary three-point docking site for O-1, O-2 and O-3 of the sulphate, respectively, and the Ala 133 peptide NH donates a hydrogen bond to the Ser 130 hydroxyl (see Fig. 2). The peptide bonds with CO groups from residues 44, 131 and 172 initiate three α-helices (Figs 1, 2*b*).

What neutralizes or stabilizes the sequestered dianion charges? Stabilization could be provided by the inducible hydrogen-bond dipoles, by the three helix macrodipoles (44–54, 131–146, 172–181) close to the sulphate (with opposite polarity) (Figs 1, 2*b*) or by a combination of the two. We propose that fixed hydrogen bond dipoles radially oriented around the sulphate, induced through polarization by the strong electric field of the dianion and further coupled (via peptide units) to several resonating or relay H-bond systems (see Fig. 2*b*), could effectively neutralize the charges. One of these systems, which is composed of two H-bonds (between O-2 of the sulphate and Gly 131 peptide NH and between Ser 130 peptide CO and Arg 134 NE), could dissipate the buried charges. Arg 134, which is 5.7 Å to the sulphate, is further involved in an inter-domain bidentate salt-link with Asp 68. Another relay system is formed by hydrogen bonding between O-4 of the sulphate and NH of Asp 11 and between CO of Tyr 10 and His 42 ND1, which is in contact with the solvent. A similar mechanism for dissipating charges could be achieved by three separate peptide bonds, each providing an NH H-bond to a sulphate oxygen and initiating via the CO group a 'zig zag' continuous sequence of alternating peptide units and H-bonds within the α-helix. The left-handed helical H-bond array has the form $O_i \cdots NH_{i+4}$, where $i = m+3(n-1)$; m is the residue bearing the first O and $n = 1, 2, 3 \cdots$. An α-helix has three such arrays; ones of interest in SBP have m values of 44, 131 and 172.

As the Ser 130 hydroxyl is polarized by the dianion and is the recipient of a hydrogen bond from 133 NH located at the N-terminus of helix 131–146, the hydroxyl proton could be shared with the O-1 of the sulphate, yielding transient HSO_4^- species (the pK_1 and pK_2 of the sulphuric acid are ~ -3 and 1.9, respectively[7]). Proton sharing could be further facilitated by the coupling of 133 NH with another resonating H-bond array ($m = 132$).

Helix macrodipoles have been implicated in anion binding at the positive N-terminus of macrodipoles[8]. It has been calculated that binding of a single negative charge 5 Å from the helix macrodipole N-terminus involves a substantial attractive energy contribution of 12 kcal mol^{-1}, which is minimally diminished by desolvation energy[8] (this value may be overestimated significantly)[9]. The three helices in the sulphate-binding protein close to the dianion could provide charge stabilization. The effect of the macrodipoles may be diminished as the sulphate is not centred on any helix axis. These helices and several others with their N-termini converging towards the binding site cleft could also promote long-range attraction of anions preparatory to actual tight binding[8]. This would seem to be generally applicable to binding proteins except that several of the substrates are uncharged sugars and amino acid zwitterions.

The mechanism we have described above confines the function of an α-helix primarily to a specific H-bonded peptide array and takes into account resonances and polarizability of the array in dissipating buried charges. The mechanism would be more effective when the array is exposed to the solvent. This is what we observed in the amphipathic[10] helices with m values of 44 and 172; the third helix is partially buried. Further, we have made similar observations from other proteins (for example, flavodoxin) known to bind anions at N-termini of amphipathic helices (unpublished findings). In this connection, it has been shown that carbonyl oxygens within the hydrophilic side of helices are often H-bonded to solvents[11].

The proposed function of a specific H-bond helical array could, in much the same way as it might enhance the sharing of a serine hydroxyl proton with the sulphate, increase the reactivity of enzyme active-site residues or of coenzymes hydrogen-bonded to the first turn of helices.

The structure of flavodoxin resembles one of the domains of the periplasmic binding proteins (G.L.G., M.A.S. and F.A.Q., unpublished data). Although the substrate site of flavodoxin is located in a crevice at the surface[12] (coordinates obtained from Protein Data Bank[13]), we observed some similarities in the binding of the phosphate moiety of the buried flavin mononucleotide with the sulphate binding described above. (1) Of the 10 H-bond donors to the phosphate oxygens, 5 are from peptide NH groups of loops and the N-terminus of the one helix (10–26) near the phosphate dianion; (2) a Ser OH simultaneously donates a H-bond to the phosphate and accepts a H-bond from a peptide NH; (3) the one line of peptide H-bonds ($m = 10$) is exposed to the solvent. As the phosphate is not deeply buried within the flavodoxin, several relay systems as found in SBP may not be required. Nonetheless, the carbonyl oxygens of peptides bearing the NH groups H-bonded to the phosphate

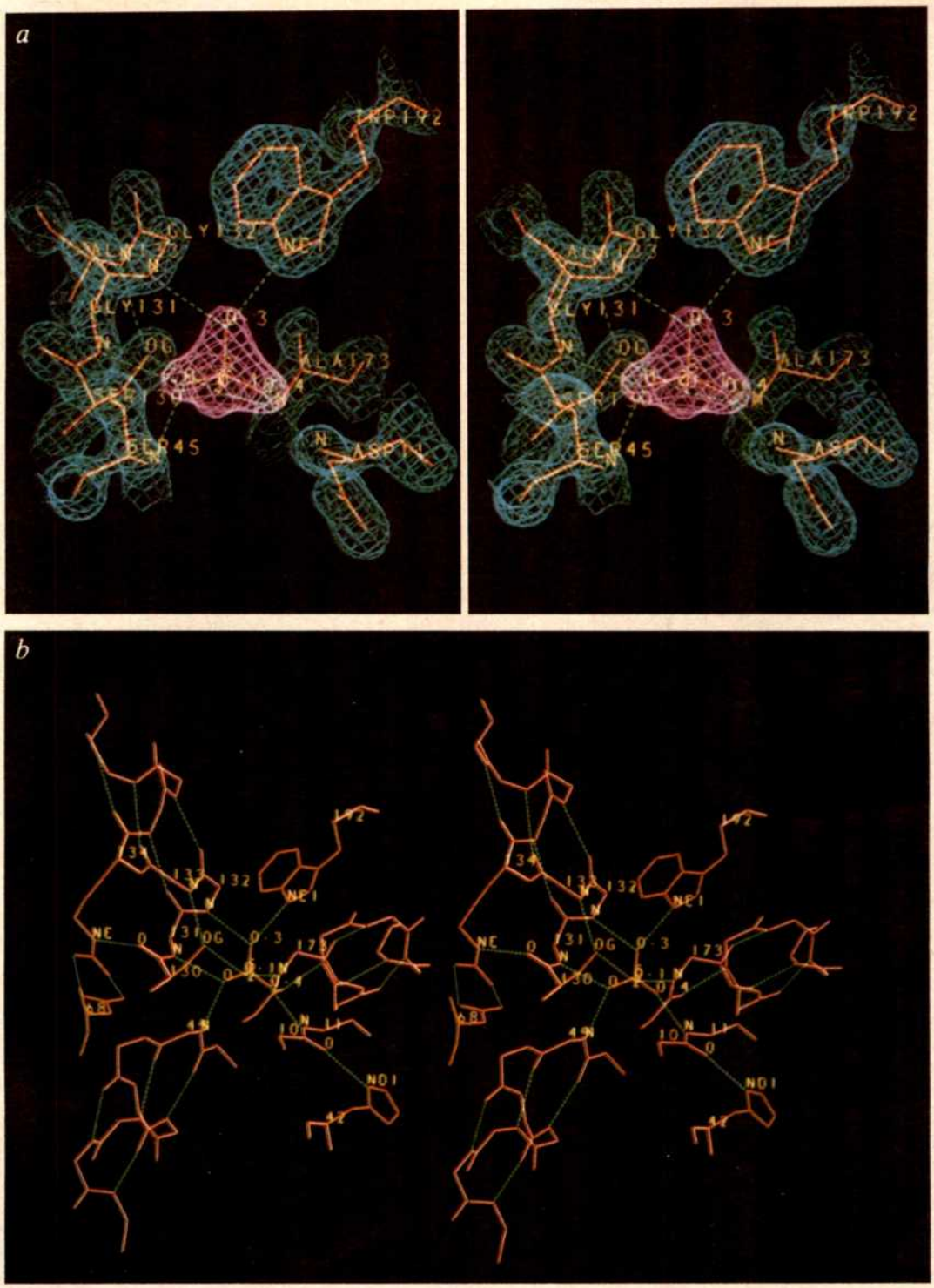


Fig. 2 *a*, Stereo view of the electron density of the tetrahedral sulphate and essential residues of the sulphate-binding protein superimposed on the refined model. The density map was calculated with coefficients $(2|F_o|-|F_c|)$ and final 2 Å refined α_c. The density surface (sulphate in purple and amino acids in blue) was contoured at 1.5 times the estimated standard deviation of the electron density and at grid-spacing of 0.4 Å. The green dotted lines represent hydrogen bonds. The H-bonds formed between the sulphate and the sulphate-binding protein are listed in Table 1. The α-carbons are identified with the amino-acid name and number. The plane formed by Ser 130 OG, Gly 131 N and Gly 132 N is parallel to and maximally superimposed on the plane of O-1, O-2 and O-3 of the sulphate. *b*, The same as *a* except that the electron density surface and residue names have been removed for clarity. Furthermore, other hydrogen-bonding systems (described in the text) and at least the first turn of three helices close to the sulphate have been added. The molecular graphics program FRODO[17] extensively modified for the E & S PS300 system[18] was used in the refinement and in generating these figures.

are in contact with the solvent.

Despite completely different substrate specificities, there are similarities in the binding sites of the L-arabinose-binding protein[1] and the sulphate-binding protein. The L-arabinose is also bound in the cleft via several H-bonds from six buried residues in both domains and is inaccessible to the bulk solvent. It is notable, especially in view of the importance of peptide bonds in anion binding, that while only side chains are involved in hydrogen-bonding the sugar in the arabinose receptor, five of the seven H-bonds to the sulphate are from the peptide backbone. (We have also observed that the charge of the solvent-inaccessible Arg 151, one of three essential charged residues in

the arabinose-binding protein, is stabilized entirely by protein and sugar dipoles[1].) As H-bonds are highly directional, they confer geometrical specificity on the site and ensure correctness of fit for the substrate. It may also be important that these bonds are stable enough to provide significant binding but are of sufficiently low strength to allow rapid ligand dissociation. The estimated turnover rates of binding proteins *in vitro* are comparable to the V_{max} values for *in vivo* transport[14]. Transport could be impeded if the sulphate were bound with salt-links.

The novel finding of general significance presented here is that charges from as strong an acid as sulphate can be stabilized in an enclosed, solvent-inaccessible site within a protein by bonds other than salt-links. This particular result and other observations reported here have far-reaching importance in understanding not only other ion-binding and transport systems, but protein stability, enzyme catalysis and other biochemical processes that require charged intermediates.

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1. Quiocho, F. A. & Vyas, N. K. *Nature* **310**, 381-386 (1984).
2. Vyas, N. K., Vyas, M. N. & Quiocho, F. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1792-1796 (1983).
3. Saper, M. A. & Quiocho, F. A. *J. biol. Chem.* **258**, 11057-11062 (1983).
4. Landick, R. & Oxender, D. L. in *Bacterial Transport* (ed. Martonosi, A. N.) 81-88 (Plenum, New York, 1982).
5. Hendrickson, W. A. & Konnert, J. H. in *Computing in Crystallography* (eds Diamond, R., Ramaseshan, S. & Venkatesan, K.) 13.01-13.23 (Indian Academy of Sciences, International Union of Crystallography, Bangalore, 1980).
6. Lee, B. K. & Richards, F. M. *J. molec. Biol.* **55**, 517-526 (1971).
7. Pauling, L. *The Nature of the Chemical Bond*, 325 (Cornell University Press, Ithaca, 1966).
8. Hol, W. G. J., van Duijnen, P. T. & Berendsen, H. J. C. *Nature* **273**, 443-446 (1978).
9. Rees, D. C. *J. molec. Biol.* **141**, 323-326 (1980).
10. Perutz, M. F., Kendrew, J. C. & Watson, H. C. *J. molec. Biol.* **13**, 669-678 (1965).
11. Blundell, T., Barlow, D., Borkakoti, N. & Thornton, J. *Nature* **306**, 281-283 (1983).
12. Smith, W. W., Burnett, R. M., Darling, G. D. & Ludwig, M. L. *J. molec. Biol.* **117**, 195-225 (1977).
13. Bernstein, F. C. *et al. J. molec. Biol.* **112**, 535-542 (1977).
14. Miller, D. M. III, Olson, J. S., Pflugrath, J. W. & Quiocho, F. A. *J. biol. Chem.* **258**, 13665-13672 (1983).
15. Isihara, H. & Hogg, R. W. *J. biol Chem.* **255**, 4614-4618 (1980).
16. Lesk, A. M. & Hardman, K. D. *Science* **216**, 539-540 (1982).
17. Jones, T. A. in *Computational Crystallography* (ed. Sayre, D.) 3303-3317 (Clarendon, Oxford, 1982).
18. Pflugrath, J. W., Saper, M. A. & Quiocho, F. A. in *Methods and Applications in Crystallographic Computing* (eds Hall, S. & Ashida, T.) 404-407 (Clarendon, Oxford, 1984).

Significance of enamel thickness in hominoid evolution

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Enamel thickness has assumed unique importance in the debate about the hominid status of *Ramapithecus*, despite the fact that there is little agreement about the meaning of 'thick enamel' or the significance of enamel thickness for hominoid taxonomy[1-19]. My aim here is to evaluate the usefulness of enamel thickness and microstructure as characteristics for determining the relationships of the later Miocene hominoids, based both on a quantitative study of enamel thickness in extant hominoids and four species of later Miocene *Sivapithecus* (including '*Ramapithecus*') and on scanning electron microscope analysis of enamel microstructure[20]. Four categories of enamel thickness are defined metrically here and have been related to enamel microstructure and developmental rates. Thin fast-formed (pattern 3) enamel represents the ancestral condition in hominoids; it increased developmentally to thick pattern 3 enamel in the great ape and human clade and that condition is primitively retained in *Homo* and in the fossil hominoid *Sivapithecus* (including '*Ramapithecus*'). Enamel thickness has been secondarily reduced in the African apes and also, although at a different rate and extent, in the orang-utan. Thick enamel, previously the most important characteristic in arguments about the earliest hominid, does not therefore identify a hominid.

The most useful measurement of the amount of enamel on a tooth would be the volume of the tissue. This could be expressed as a thickness by dividing the volume of enamel by the surface area of the enamel/dentine junction over which the enamel formed; this area is proportional to the number of ameloblasts which formed the enamel. Such a measurement can be approximated by dividing the area of the enamel cap exposed by a single saw cut through the tooth by the length of the enamel/dentine junction over which it formed (average enamel thickness, Fig. 1). Single linear measurements of enamel thickness were found to be poorly correlated with this value, and such studies[15], especially those based on estimates from worn teeth[4] or naturally fractured teeth[21,22], are of limited value[20].

Enamel thickness increases allometrically (with increasing body and tooth size) in anthropoid primates[4,15]. Gorillas have absolutely thicker enamel than do chimpanzees (Fig. 1) in direct relation to body size[20], whereas orang-utans and humans have relatively thicker enamel when scaled for body and tooth size (Fig. 1)[20]. As body weight data are not available for fossil species, a dental estimate of body size must be used to scale average enamel thickness. Tooth crown measurements are of little use[4] as they include two measurements of enamel thickness in their magnitude and are not independent variables. The best estimator of body size was the combined area of dentine and pulp in the same section on which enamel thickness was measured (see Fig. 1)[20]. When combined with average enamel thickness (c/e), this produces a dimensionless index: relative enamel thickness $= (c/e \times 100)/\sqrt{b}$, where c is the area of the

Table 1 Relative enamel thickness of fossil and recent great apes

	Pan	*Gorilla*	*Homo*	*Pongo*	*Sivapithecus*
No. of specimens	14	17	13	17	9
Mean	10.10	10.04	22.35	15.93	19.71
Minimum	7.02	6.75	13.76	11.32	16.07
Maximum	13.31	13.39	32.26	20.45	22.68
Standard deviation	2.09	1.74	6.23	2.51	2.58
Mean low 95%	8.90	9.15	18.58	14.65	17.73
Mean high 95%	11.30	10.93	26.12	17.21	21.69
$\frac{c/e}{\sqrt[3]{\text{b.wt}}} \times 100$ (mean)	1.68	1.80	3.00	2.84	—

The sample studied included 24 unworn, or very lightly worn, molar teeth for each of *Pan troglodytes* (the chimpanzee), *Gorilla gorilla*, *Pongo pygmaeus* (the orang-utan) and *Homo sapiens*, and a smaller number of molars of *Hylobates*, *Sivapithecus sivalensis* (formerly called *Sivapithecus indicus*[20,28]), *Sivapithecus punjabicus* (formerly '*Ramapithecus*' *punjabicus*[14,20]), *Sivapithecus darwini* and *Sivapithecus alpani* (formerly referred to '*Ramapithecus*' *wickeri*[20,22]). These were mainly cut longitudinally in the coronal plane through the mesial cusps to provide two surfaces for measuring enamel thickness. Examples of each species were sectioned in the sagittal plane to determine the correspondence of the tips of the dentine horns with the cusp tips. The teeth were cut using a diamond wafering machine (Isomet, Buehler Ltd) producing a cut 350 μm wide. It has been shown that obliquity of section can exaggerate apparent enamel thickness[20], but the method used here ensures that measurements of enamel thickness were taken at or within a few micrometres of the ideal plane of section through the enamel cap, and even at worst no more than 175 μm mesial or distal to the ideal (see Fig. 1 legend). Mean low 95% and mean high 95% refer to the 95% confidence limits for the mean values for these samples. The final row gives values, for the living species, of a relative enamel thickness index based on body weight (b.wt, data from Dr Ben Rudder), which provides the same ranking of enamel thickness.

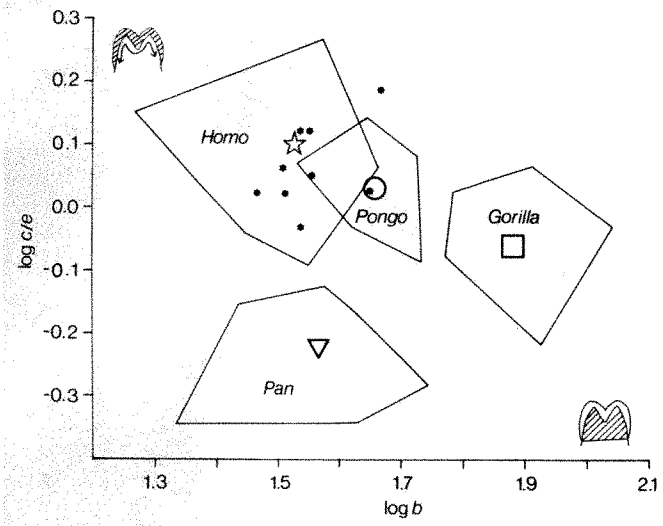


Fig. 1 Enamel thickness in relation to size. The vertical axis shows the average enamel thickness (c/e) and the horizontal axis the combined dentine/pulp area (b); both axes are logarithms to base 10. The shaded area of the tooth section shown on the y-axis is the enamel cap area (designated c) and the length of the interface between the shaded area and the unshaded area on the y-axis is the enamel/dentine junction length (e): average enamel thickness = c/e. The shaded area of the tooth section on the x-axis is the combined area of dentine and pulp (b), which was found to be the best dental approximator for body size that excluded any contribution from the enamel[20]. The species ranges observed are shown as minimum area polygons, with the species means indicated. Fossil specimens attributed to four species of *Sivapithecus* are shown by site, ☆ representing specimens from the Siwaliks, Indo-Pakistan, and * representing specimens from Pasalar, Turkey.

Sample details are given in Table 1 and individual data points are given in ref. 20. Measurements for all upper and lower molars are combined, which could only increase the species ranges, as no significant differences in enamel thickness between different molars were found[20]. Measurements were taken on photographs of the cut surface taken at constant magnification, with the cut face parallel with the film plane, and prints were also made at constant magnification[20]. Area and perimeter measurements were made using a pen transducer attachment for the graphics pad of an Apple computer, and tracing errors were small[20]. Linear measurements were taken to determine a good simple approximator of average enamel thickness. Correlations between average enamel thickness (c/e) and linear enamel thicknesses were $r = 0.27$–0.75 for nine single linear dimensions in *Gorilla*[20]; when two or three of these measurements were averaged before calculating the correlation, it increased to $r = 0.74$–0.76 (ref. 20); and the correlation for the mean of all nine measurements on c/e was 0.85 (ref. 20). These results indicate the unreliability of single linear dimensions (*contra* Gantt[15]), and raise questions about the reliability of studies based on these[15,19], and even more so about studies based on estimates of linear dimensions of enamel thickness[4,21].

enamel cap, e is the length of the enamel/dentine junction over which the enamel formed and b is the combined area of dentine and pulp in the same section (Fig. 1). Table 1 gives values for this index together with values for a second index expressing enamel thickness relative to the cube root of body weight; the two dimensionless indices produce the same ranking of relative enamel thickness, although their actual values differ[20]. The dentally based index has therefore been used as a measure of relative enamel thickness which is applicable to fossil species.

Four categories of relative enamel thickness are defined, based on the 95% confidence limits of the species means of relative enamel thickness (Table 1): thin enamel (species mean values of relative enamel thickness 8.90–11.30), intermediate/thin enamel (mean values 11.31–14.64), intermediate/thick enamel (mean values 14.65–17.49), and thick enamel (mean values 17.50–26.20). Thin enamel is found in *Pan*, *Gorilla* and *Hylobates*, intermediate/thick enamel in *Pongo* and thick enamel in *Homo*, and also in *Sivapithecus* (17.73–21.69).

These variations in enamel thickness can be explained in terms of the microstructure of hominoid enamel. Pattern 1 enamel[23] has previously been reported to occur in great ape enamel, while pattern 3 enamel is found in hominines[24]. In contrast, Vrba and Grine[25] found pattern 3 enamel to be common in all hominoids, and Boyde and Martin[26] found that pattern 3 enamel predominates in the deep layers of hominoid enamel, sampled in developing surface preparations, although some pattern 1 enamel is always present superficially. The prevalence of pattern 3 enamel in hominoids has subsequently been recognized by Gantt[19].

In the present work, sequential cuts made into the enamel provided information about enamel prism cross-sections, at a series of known depths, in the course of sectioning teeth for enamel thickness measurements[20,26]. The cut surfaces (Fig. 2*C*) were examined by back-scattered scanning electron microscopy to give additional information about packing patterns in the deeper enamel, prism cross-striation repeat intervals, and the distribution of Hunter-Schreger bands (Fig. 2)[20,26,27].

The distributions of these features were correlated in hominoid enamel. In all cases, pattern 3 enamel was associated with a cross-striation repeat interval of 5–7 μm (Fig. 2*B*), corresponding to the daily rate of ameloblast secretion (values are averages for large numbers of repeat intervals)[20]. Hunter-Schreger bands occurred only in areas of pattern 3 enamel (Fig. 2*A*). Where pattern 1 enamel was found, it was associated with cross-striation repeat intervals of <2.5 μm, a much lower accretionary rate, and an absence of Hunter-Schreger bands. This indicates that, in hominoids, pattern 3 enamel with prism decussation is formed about three times faster than is non-decussating pattern 1 enamel.

All of the hominoid species examined had two very thin (20–30 μm) layers of slowly formed (pattern 1) enamel, one at the tooth surface and the other immediately adjacent to the enamel/dentine junction, corresponding to the start and finish of enamel production by the ameloblasts. In *Homo*, *Hylobates* and *Sivapithecus*, the rest of the enamel was fast-formed (pattern 3) decussating enamel (Fig. 2) accounting for 90–95% of the enamel thickness.

Gibbons have thin enamel because it forms for a relatively shorter period (in relation to size) than does enamel in species of the great ape and human clade, which have extended developmental periods for tooth enamel relative to size. If all of their enamel was fast-formed it would be thick, as indeed it is for man. In the great apes, the thin enamel and intermediate/thick enamel form over the same relative time period as in the thick-enamelled species, but their enamel is thinner because the superficial layer of slow-formed pattern 1 enamel is much thicker and the average daily incremental rate is reduced. In *Pongo*, the slowly formed layer represents ~20% of maximum enamel thickness (~200 μm laid down at 2.5 μm per day and a further 50 μm laid down at 1.8 μm per day). In *Pan* and *Gorilla*, the slowly formed layer represents ~40% of maximum enamel thickness (~400 μm in *Gorilla* and ~300 μm in *Pan*), and was formed throughout at <1.5 μm per day. Thin enamel is structurally different in gibbons and African apes, and therefore not homologous, and the slowing down of enamel secretion is similarly non-homologous between the orang-utan and the African apes[20].

Thin enamel is a developmentally heterogeneous category and is therefore subdivided into two: first, thin enamel, all of which is formed at the fast (pattern 3) rate (termed thin, fast), present in gibbons; and second, thin enamel, a substantial portion of which is formed at the slow (pattern 1) rate (termed thin, slowed), present in the African apes. The intermediate/thin and the intermediate/thick categories could also be developmentally heterogeneous and are similarly subdivided. Thick enamel has been found only in species in which all of the enamel was formed at the fast (pattern 3) rate, and is therefore unlikely to have been developmentally heterogeneous in hominoids (Fig. 3*a*). This study therefore provides seven relevant categories of

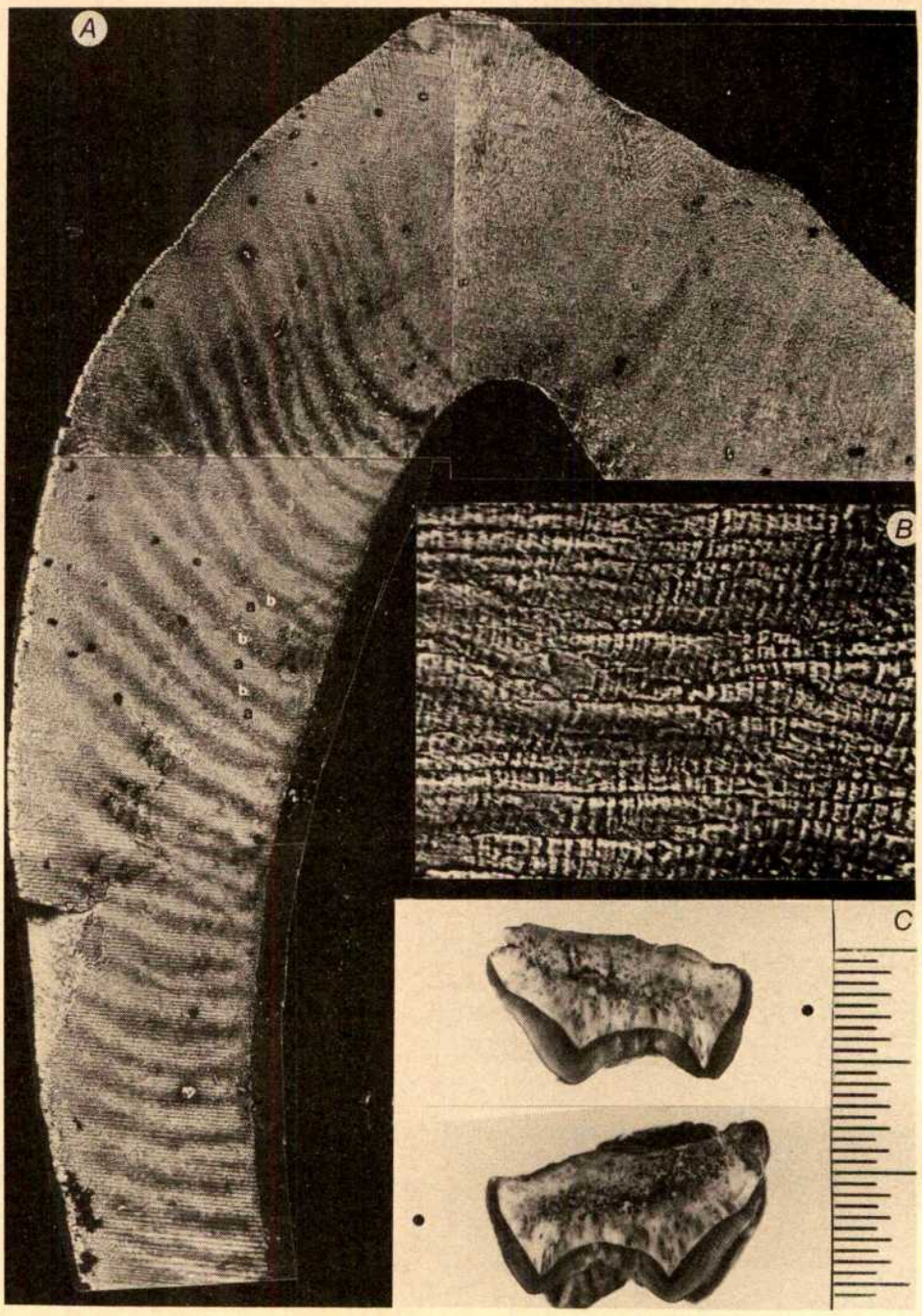


Fig. 2 Enamel thickness and structure in extant and extinct Hominoidea. ***A***, Montage of the enamel of the lingual cusp of a lower second molar of *Sivapithecus alpani* (BP 13) from Pasalar, Turkey (~14 Myr BP), with the enamel/dentine junction to the right. The cut face was lightly polished with 1-μm diamond and then etched with 0.5% H_3PO_4 for 30 s. Hunter-Schreger bands extend across most of the enamel thickness, even where it is thickest. Only the region above the tip of the dentine cusp has no prism decussation. Parazones (a) and diazones (b) are labelled and result in the Hunter-Schreger band pattern. Field height, 4.27 mm. ***B***, Cross-striations in a slowly formed region of the outer lingual enamel in a *Pongo pygmaeus* upper second molar. Cervical is towards the top, tooth surface to the left. The specimen was prepared by diamond polishing the cut face to a 1-μm finish and then etching it with EDTA *p*H 7.2 for 18 h to remove the smeared enamel and to reveal the enamel prism boundaries and cross-striations. The micrograph is a back-scattered electron image[26,27] which produces atomic number contrast, that is, a density-dependent image, when topographic relief is low. Field width, 146 μm. ***C***, Coronal longitudinal section through the mesiodistal cusps of a maxillary first molar (M^1) of *S. sivalensis* (M 13365). The upper section shows the anterior face revealed by the saw cut and the lower section the posterior face (350 μm apart); the buccal side of the tooth is indicated by a black spot. The scale bar is marked with 0.5-mm divisions. Some variation in any enamel thickness data will be the result of imperfect measurements and imperfect sectioning. Oblique sections act to increase the apparent enamel thickness[20], and this effect has been minimized here by taking minimum, and therefore more nearly radial, measurements (of enamel thickness) from the two cut faces in conjunction with maximum values for all dentine/pulp measurements[20]. When the saw cut has passed through the maximum diameter of the dentine horns, minimum values from both faces are used for enamel dimensions and maxima for dentine/pulp. When the saw cut has missed the plane of the maximum diameter of the dentine cusps (as with this *S. sivalensis* specimen), one face will be closer to the ideal plane of measurement than will the other[20]. The posterior face of M 13365 lies closest to the ideal plane of section, as it produces minimum values for all enamel thickness measurements and maximum values for all dentine/pulp measurements, and was used for measurement.

enamel thickness/formation for assessment in fossil species, in comparison with the two ('thick enamel' and 'thin enamel') used previously[7,8,13-15,17-19,22].

Figure 3*b* shows the conditions of enamel thickness and formation rates in the common ancestors of the various hominoid clades as interpreted from the data presented here. They provide the basis for the assessment of the relationships of *Sivapithecus* and *Dryopithecus* (see Fig. 3*b* legend).

Thick-enamelled, human-like teeth have previously been used to support the hominine status of *Ramapithecus*[1,17,18] and more recently *Sivapithecus*[4-8] and even *Pongo*[9], but these features are interpreted here to be primitive within the great ape and human clade (Fig. 3*b* legend). Thick enamel, having been considered the most important character in the assessment of the affinities of Miocene hominoids, turns out to be the least diagnostic of the enamel thickness categories and of no relevance to the taxonomic placement of *Sivapithecus* (including '*Ramapithecus*') within the great ape and human clade[20].

Thick pattern 3 enamel does not define a hominid. Moreover, the common ancestor of the great apes and man, and of the African apes and man, would have had teeth resembling those of hominids. This should be considered when attempting to establish the earliest record of hominids from deposits of later Miocene to early Pliocene age. Of the living members of the great ape and human clade, only *Homo sapiens* retains the condition of enamel thickness and development from the common ancestor of that clade (Fig. 3*b*) and may therefore be regarded as the most dentally primitive member of it.

This work was carried out in the Department of Palaeontology, British Museum (Natural History) and the Departments

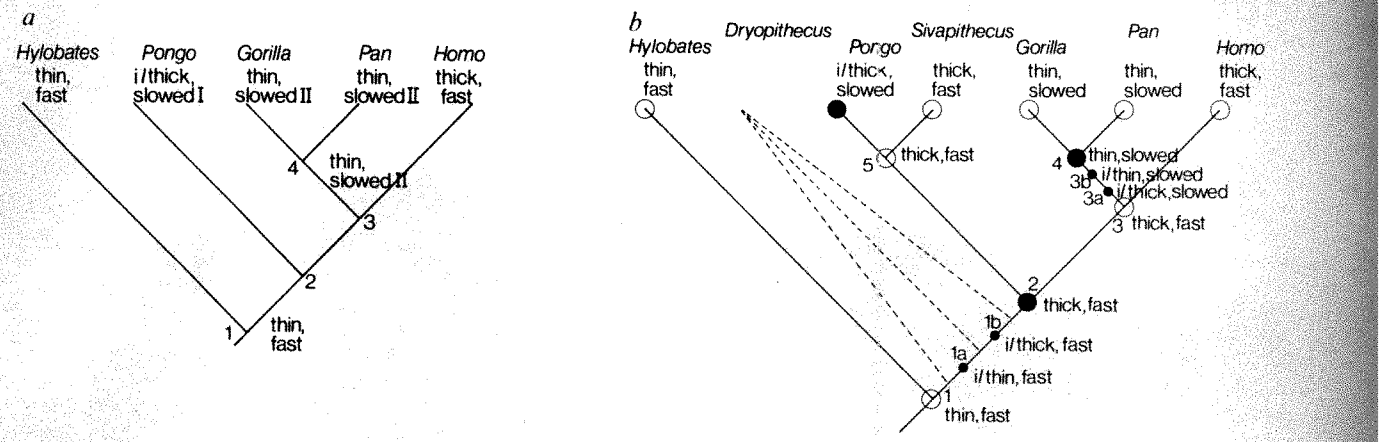


Fig. 3 Interpretations of the evolution of hominoid enamel. *a*, Enamel thickness and formation rates in extant great apes and humans; *b*, positions of *Sivapithecus* and *Dryopithecus*. The relationships among the living members of the great ape and human clade are based on cladistic interpretation of overall morphological pattern[3-8,10-20,22,28,29]. The recently proposed close relationship between human and orang-utan[9] depends very largely on the interpretation of thickened enamel as a shared derived character between these two genera, but as this has not been shown to be the case, and as our main aim here is to determine ancestral conditions, this view is not accepted here. *a*, Thin enamel predominates among cercopithecoids, platyrrhines, tarsiers and strepsirhines[4,15,18] and in *Hylobates*, so that it is parsimonious to interpret the condition in the last common ancestor (LCA) of the Hominoidea as being with thin, fast-formed (pattern 3) enamel (node 1), with this condition being retained in extant gibbons. Similarly, because both *Pan* and *Gorilla* have thin slowed-down enamel, it is likely that they retained this condition from their common ancestor (node 4). The situation for the LCA of the great ape and human clade (node 2) and for other contained clades is less clear. There are seven possibilities for the condition of enamel thickness/development at node 2 (see text). On the grounds that: (1) the thin enamel in gibbons and African apes is not homologous; (2) the slowed-down enamel in the African apes and the orang-utan is not homologous; and (3) the slowed-down pattern 1 enamel is developmentally derived from pattern 3 type with a relatively long total developmental period, in hominoids, the most parsimonious explanation for the distribution of enamel thickness and development among extant great apes and humans is that of thick enamel all of which formed at the fast, pattern 3 rate in the LCA of the great ape and human clade. *b*, This interpretation has considerable implications for the placement of fossil hominoids based on enamel thickness and structure. *Sivapithecus* is shown according to facial morphology[13,14,16,20,28,29], and this position is in accordance with enamel thickness data. Fossil taxa that do not have thick enamel could belong to several positions in hominoid phylogeny. If any of these taxa should be found to have either intermediate/thin or intermediate/thick fast formed enamel, it could be placed as the sister group of the great ape and human clade (nodes 1a, 1b). *Dryopithecus* is shown placed according to its possession of other derived characters shared with the great ape and human clade[20,29]. Its position could be more completely determined if it were known whether *Dryopithecus* had thin, intermediate/thin or intermediate/thick enamel. Closed circles at nodes indicate derived conditions with respect to the previous ancestral node; open circles indicate nodes at which the ancestral condition is retained primitively. According to this interpretation, the common ancestor of the Hominoidea (node 1) had thin enamel all of which formed at the fast, pattern 3 rate. The common ancestor for the great ape and human clade (node 2) had thick enamel, all of which was formed at the fast, pattern 3 rate. Nodes 1a and 1b represent hypothetical common ancestors at probable stages of enamel evolution, node 1a with intermediate/thin enamel and node 1b with intermediate/thick enamel, all of which formed at the fast, pattern 3 rate. No living hominoids display these conditions, but we might expect to find these derived conditions in fossil hominoid species from the middle or late Miocene (such as *Dryopithecus* and/or *Kenyapithecus*). Thick, fast-formed pattern 3 enamel is primitively retained at node 3, the common ancestor of the African ape and human clade, and node 5, the common ancestor of *Sivapithecus* and the orang-utan. This condition is modified in the *Pongo* portion of the clade, with a secondary reduction to intermediate/thick enamel. This stage should be detectable in fossil pongines from the later Miocene or Pliocene or Pleistocene. Thick, fast-formed enamel is also primitively retained in the exclusively hominine clade. The common ancestor of the African apes (node 4) had thin enamel, much of which formed at a reduced daily rate and which is therefore interpreted as being secondarily reduced. Nodes 3a and 3b represent hypothetical ancestors of the African apes at stages of enamel evolution through which they must have passed.

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1. Simons, E. L. *Postilla* **57**, 1-9 (1961).
2. Ciochon, R. L. & Corruccini, R. S. (eds) *New Interpretations of Ape and Human Ancestry* (Plenum, New York, 1983).
3. Ciochon, R. L. in *New Interpretations of Ape and Human Ancestry*, 783-843 (Plenum, New York, 1983).
4. Kay, R. F. *Am. J. phys. Anthrop.* **55**, 141-152 (1981).
5. Kay, R. F. *Proc. natn. Acad. Sci. U.S.A.* **79**, 209-212 (1982).
6. Kay, R. F. *Int. J. Primatol.* **3**, 113-173 (1982).
7. Kay, R. F. & Simons, E. L. in *New Interpretations of Ape and Human Ancestry*, 577-624 (Plenum, New York, 1983).
8. de Bonis, L. in *New Interpretations of Ape and Human Ancestry*, 625-649 (Plenum, New York, 1983).
9. Schwartz, J. H. *Nature* **308**, 501-505 (1984).
10. Zihlman, A. H. & Lowenstein, J. M. in *New Interpretations of Ape and Human Ancestry*, 677-694 (Plenum, New York, 1983).
11. Wolpoff, M. H. in *New Interpretations of Ape and Human Ancestry*, 651-676 (Plenum, New York, 1983).
12. Greenfield, L. O. in *New Interpretations of Ape and Human Ancestry*, 695-703 (Plenum, New York, 1983).
13. Andrews, P. & Tekkaya, I. *Palaeontology* **23**, 85-95 (1980).
14. Andrews, P. & Cronin, J. E. *Nature* **297**, 541-546 (1982).
15. Gantt, D. G. thesis, Washington Univ. (1977).
16. Ward, S. C. & Pilbeam, D. R. in *New Interpretations of Ape and Human Ancestry*, 211-238 (Plenum, New York, 1983).
17. Simons, E. L. & Pilbeam, D. R. in *The Functional and Evolutionary Biology of the Primates*, 36-62 (Aldine, Chicago, 1972).
18. Simons, E. L. *J. hum. Evol.* **5**, 511-528 (1976).
19. Gantt, D. G. in *New Interpretations of Ape and Human Ancestry*, 249-298 (Plenum, New York, 1983).
20. Martin, L. B., thesis, London Univ. (1983).
21. Beynon, A. D. & Wood, B. A. *J. dent. Res.* **63**, 505, abstr. 144 (1984).
22. Andrews, P. & Tobien, H. *Nature* **268**, 699-701 (1977).
23. Boyde, A., thesis, London Univ. (1964).
24. Gantt, D. G., Pilbeam, D. R. & Steward, G. P. *Science* **198**, 1155-1157 (1977).
25. Vrba, E. S. & Grine, F. E. *Science* **202**, 890-892 (1978).
26. Boyde, A. & Martin, L. *Anat. Embryol.* **165**, 193-212 (1982).
27. Boyde, A. & Jones, S. J. *Anat. Embryol.* **168**, 211-226 (1983).
28. Martin, L. & Andrews, P. *Cour. Forsch. Inst. Senckenberg* **67**, 25-40 (1984).
29. Martin, L. & Andrews, P. *Primate Eye* **18**, 4-7 (1982).
30. Boyde, A. & Martin, L. in *Tooth Enamel* Vol. 4, 417-421 (Elsevier, Amsterdam, 1984).

Atrial natriuretic factor— a circulating hormone stimulated by volume loading

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The cardiocytes of mammalian cardiac atria contain granules very similar to those in endocrine cells[1,2]. The number of these atrial granules is related directly to salt loading and blood volume[3]. Furthermore, crude extracts of rat atria and granule preparations have powerful natriuretic and diuretic effects[4,5]. These effects are mediated by peptides identified previously as atrial natriuretic factor (ANF). The peptides are derived from a common precursor, whose structure has been elucidated recently[6–15]. Although there is indirect evidence from morphological studies that at least some of these peptides may be released into the blood and function as hormones, their presence in the blood has not yet been demonstrated. Here we describe a sensitive and specific radioimmunoassay for ANF and its stimulation on volume loading.

The rat synthetic peptides used here were the 21-amino acid atriopeptin I (AP I), the 23-amino acid atriopeptin II (AP II) and the 24-amino acid atriopeptin III (AP III)[8]. To obtain antibodies, New Zealand White rabbits were injected with AP II coupled to bovine thyroglobulin. AP III, which corresponds to the sequence of AP II extended at the C-terminus by a tyrosine residue, was iodinated with ^{125}I as a tracer. The radioimmunoassay for ANF was performed at a final antibody dilution of 1:80,000 and AP III was used for the construction of standard curves. All concentrations of ANF-like immunoreactive material were expressed as weight equivalents of AP III (see Fig. 2 legend).

When extracts from rat atria prepared as described in Fig. 1 legend were examined by radioimmunoassay, the mean concentration of ANF-like immunoreactive material was 86.9 ± 14.3 ng mg^{-1} wet weight. Serial dilution of the extracts inhibited the binding of tracer to anti-ANF antiserum in parallel with the standard curve of ANF III.

Analysis of atrial extracts by HPLC revealed several immunoreactive peaks (Fig. 1*a*). Small amounts of ANF-like material were detected in the fractions where the synthetic standards eluted, but neither of the two major peaks corresponded to the position of these standards. Most of the immunoreactive material eluted with the upper third of the gradient, indicating a higher lipophilicity. The major peak appeared with a retention time of 54 min. Subsequent molecular mass sieving studies (not shown) indicated that this material represents higher relative molecular mass (M_r), consistent with the idea that it may represent precursor peptides. We studied the release of ANF-like material from rat atria *in vitro* using a modification of the isolated perfused heart preparation[16]. The heart was perfused with constant pressure through the aorta and buffer added to the right atrium via the upper vena cava. Increasing the perfusion rate gave a rise in right atrial pressure, monitored by a pressure transducer connected to a cannula in the lower vena cava. When right atrial pressure was raised by 1 mm Hg, the rate of ANF-like material release into the perfusate was doubled. A several-fold increase was observed after elevation of the pressure by 5 mm Hg (Table 1).

Figure 1*b* shows a HPLC–radioimmunoassay analysis of the perfusate collected at 5 mm Hg right atrial pressure. In contrast to the distribution of ANF-like material on HPLC of atrial extracts, the major fraction eluted with a retention time between that of AP II and AP III, whereas little high-M_r material was present. Muscle stretch is therefore a strong stimulus for liberation of ANF-like immunoreactive material from atria; small ANF peptides rather than precursor molecules are released.

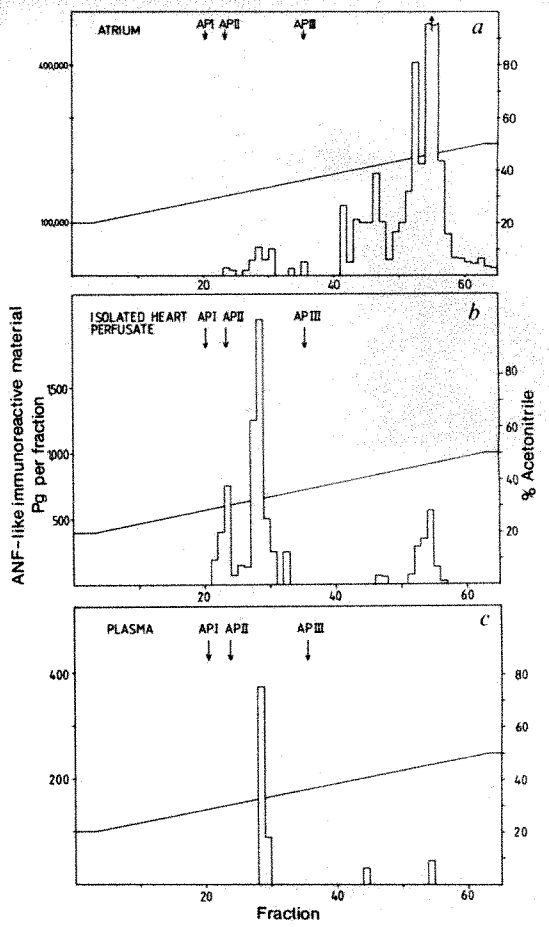


Fig. 1 Characterization of ANF-like immunoreactive material in: *a*, atrial tissue extracts; *b*, perfusate from isolated perfused rat heart; *c*, plasma, by reverse-phase HPLC using a Waters 2 Pump system and BondaPak C 18 column (40 cm × 1 cm). A linear gradient elution system was used from 20 to 50% over 60 min; solvent A, 0.01 M NH_4-trifluoroacetic acid (TFA) in water; solvent B, 0.01 M NH_4-TFA in acetonitrile; flow rate 1.0 ml min^{-1}. Fractions (1.0 ml) were collected, lyophilized and assayed for ANF-like material by radioimmunoassay (see Fig. 2 legend). Arrows indicate the elution positions of the synthetic peptides AP I, AP II and AP III, respectively. The recovery of these peptides was between 85 and 90% as determined by radioimmunoassay.
Methods. *a*, Separation of extracts from rat atria. For extraction the tissue was minced and boiled for 15 min in 0.1 M HCl to avoid proteolysis. The cooled tissue was homogenized subsequently using a Polytron tissue homogenizer and centrifuged for 30 min at 3,000 *g*. The resulting supernatant was extracted using ODS-silica cartridges as described for plasma in Fig. 2 legend, and subjected to HPLC. *b*, Analysis of ANF-like material in the perfusion buffer from the isolated perfused rat heart (see Table 1). Perfusate was collected during pressure stimulation of the right atria (5 mm Hg) and extracted by using ODS-silica cartridges before application to the HPLC column. *c*, Characterization of rat plasma ANF-like material. After insertion of chronic catheters into the femoral vein, rats were anaesthetized with pentobarbital and loaded with 8 ml normal saline as described in Fig. 2 legend. Blood was withdrawn from the aorta after laparotomy, centrifuged and plasma-extracted by passing through ODS-silica cartridges according to the procedure in Fig. 2 legend. The material subjected to HPLC corresponded to 2 ml plasma. Note that the retention time of the peak fraction is identical to that from the perfusate in *b*.

ANF-like material was also found in rat plasma. When extracted according to the procedure described in Fig. 2 legend, the mean plasma concentration in barbiturate-anaesthetized rats was 54.6 ± 13.9 pg ml^{-1} (mean ± s.d., $n = 10$). Plasma levels showed marked and dose-dependent increases on volume expansion (Fig. 2). Infusion of 2 ml normal saline produced a

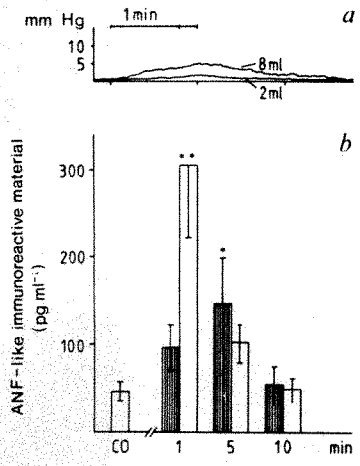


Fig. 2 Influence of volume expansion on ANF-like material in rat plasma. Values are expressed as weight equivalents of AP III (mean ± s.d., $*P < 0.05$; statistical analysis by Bonferoni test). Seventy male Wistar rats (370–420 g) in which catheters had been inserted in the femoral vein, were divided into seven groups of 10 animals. Three groups received either a 2-ml (*b*, shaded columns) or 8-ml (*b*, open columns) saline infusion i.v. under pentobarbital anaesthesia. The remaining group served as a control. Infusion time 1 min. Changes in right atrial pressure during volume load were measured in four animals by means of a catheter inserted into the right atrium through the jugular vein. *a*, Two representative traces.

Methods. Preparation of blood samples: Following laparotomy, blood was taken from the aorta either 1, 5, or 10 min after termination of volume load into plastic syringes containing aprotinin (1,000 kallikrein inactivator units ml^{-1}), soybean trypsin inhibitor (100 α-*N*-benzoyl-L-arginine ethyl ester units ml^{-1}) and EDTA (1 mg ml^{-1}). Plasma was separated by centrifugation and processed for extraction of ANF-like material as follows: 2 ml of each sample were diluted with 3 vol of 4% acetic acid and after centrifugation pumped at a rate of 4 ml min^{-1} through a small column containing ODS particles (Porasil; Machrey and Nagel). After a wash with 5 ml water, the absorbed peptides were eluted with 86% ethanol in 4% acetic acid. After evaporation and lyophilization, the dry residue was dissolved in radioimmunoassay buffer for determination of ANF-like material. Radioimmunoassay of ANF-like material: Synthetic rat AP II (Bachem) was used for immunization. The peptide was coupled to bovine thyroglobulin using carbodiimide[21] and 100 μg of the conjugate in 1 ml saline were emulsified with the same volume of complete Freund's adjuvant and injected intradermally at multiple sites on the backs of NZW rabbits. The first four immunizations were given at weekly intervals; subsequent booster injections were made every month. Rat AP III was used for labelling. Iodination with ^{125}I was performed according to the chloramine-T method[22]. For separation of the labelled peptide from free iodine, the reaction mixture was passed through ODS-silica cartridges and eluted as described above including a wash with 10% ethanol in 4% acetic acid before the final elution step. The specific activity of the tracer was 80–100 μCi μg^{-1}. The radioimmunoassay was performed in 0.1 M Tris buffer, *p*H 7.4, containing 0.1% gelatine. Synthetic AP III (1.95–1,000 pg per tube) was used to construct standard curves. The incubation mixture consisted of 0.2 ml buffer, 0.1 ml standard or sample and 0.1 ml antibody (final dilution 1:80,000). Tracer (4,000 c.p.m. per tube) was added after 48 h at 4 °C. After an overnight second incubation, separation of free from antibody-bound ^{125}I-peptide was achieved by adding 0.2 ml dextran-coated charcoal. Using this procedure, the lowest concentration of AP III yielding a binding significantly different from that in the absence of standard at the 95% confidence interval, was 2 pg per tube. The 50% intercept was at 41 pg per tube. The inter-assay variation was 14.1% ($n = 6$), and the intra-assay variation was 14.1% ($n = 6$), and the intra-assayy variation 7.1% ($n = 10$). A complete cross-reaction was observed with AP I and AP II. The antibodies did not (<0.001%) cross-react with the following peptides: angiotensin I and II, corticotropin-releasing factor, adrenocorticotropin, oxytocin, vasopressin, β-endorphin, α-neo-endorphin, dynorphins of different chain lengths, enkephalins, neuropeptide Y, somatostatin, vasoactive intestinal polypeptide. For studies on the recovery of ANF from plasma, various dilutions of synthetic AP III were added to plasma treated with charcoal for elimination of endogenous peptides. Using the ODS cartridges procedure for extraction, the recovery of synthetic AP III ranged from 62 to 70% both in the upper and lower ranges of the standard curve. The serial dilutions of extracts from rat plasma as well as atrial tissue and heart perfusate inhibited the binding of tracer to antibody in parallel with the standard curve of AP III.

:wo- to threefold rise in plasma ANF-like material. Administra-ion of 8 ml, corresponding to a ~30% volume expansion, gave i sixfold increase. The peaks in right atrial pressure in these experiments were 1–1.5 and 5 mm Hg, respectively. Similar esults were obtained in experiments using whole blood obtained rom Wistar rats of the same age and sex. In these conditions the peak values appeared at 6 min after infusion (data not shown).

For characterization of the ANF-like immunoreactive material in plasma, the blood volume of five rats was expanded by intravenous (i.v.) administration of 8 ml saline. The animals were bled 1 min after the infusion, the blood was pooled, the plasma extracted as described in Fig. 2 legend and subjected to HPLC. Radioimmunoassay revealed one major peak between the positions of AP II and AP III (Fig. 1*c*), corresponding exactly to the major peptide in the heart perfusate (Fig. 1*b*). In addition, very small amounts of ANF-like material were detected in the position of the high-M_r peptides of atrial extract. When the addition of aprotinin and soybean trypsin inhibitor to the blood was omitted, the peak fraction of the plasma HPLC was shifted to the position of AP III, indicating that degradation by plasma enzymes affects ANF metabolism.

This represents the first time that ANF has been demonstrated and characterized in the blood, although many attempts have been made to identify a natriuretic hormone that could increase renal excretion of salt and water[17,18]. Material having biological natriuretic activity has been reported in the blood and urine of several species[19] and its concentration was found to increase when the volume of blood or extracellular fluid was expanded experimentally[17–20]. Our HPLC–radioimmunoassay data show for the first time that rat plasma contains a substance immunologically related to the natriuretic peptides in and released from rat atria[6–15]. The plasma concentration is increased by physiological stimuli such as volume expansion.

Cardiac tissue contains many natriuretic peptides derived from the same precursor molecule. It is not yet known which of these are extraction artefacts and which occur naturally. In the plasma, we found a single peptide identical to the one released from the heart. Plasma ANF is closely related to but not identical with AP I, AP II and AP III, provided there is adequate protection from enzymatic degradation, in accordance with the suggestion that these peptide fragments would not occur in the heart if proteolysis was prevented during the extraction procedure from the atria[10]. Elucidation of the nature of circulating ANF will be possible when more ANF peptides have been synthesized and are available as reference substances. The knowledge of the exact amino-acid sequence is probably not crucial, as the peptides so far described usually exhibit variations in length of the amino-terminal region, which seem not to affect their biological function.

The elution pattern of ANF-like immunoreactive material obtained from atrial extracts indicates that most of it is not identical to the plasma peptide, but probably represents storage

Table 1 Effect of mean right atrial pressure on the rate of release of ANF-like immunoreactive material from the isolated perfused rat heart (pg min^{-1})

Expt no.	Change in right atrial pressure: Control	1 mm Hg	5 mm Hg
1	768	972	—
2	575	740	—
3	533	883	—
4	585	—	1,763
5	345	—	1,250
6	408	—	845

Rat hearts were removed and perfused through the aorta with Krebs/Ringer's solution at a constant pressure (122 mm Hg). The upper vena cava was cannulated and buffer infused into the right atrium at various rates to increase the mean right atrial pressure by either 1 or 5 mm Hg. The atrial pressure was monitored continuously by a pressure transducer connected to a catheter inserted into the lower vena cava. Mean atrial pressure varied between −0.5 and +0.2 mm Hg under basal conditions. Perfusate was collected and 1-min fractions extracted using the octadecasilyl (ODS)-silica cartridges technique for the radioimmunological determination of ANF-like immunoreactive material. The 5 mm Hg perfusate was used for HPLC characterization (see Fig. 1*b*).

forms of ANF serving as biosynthetic precursors for the ANF released into the blood. Our data demonstrate that, on stimulation, small concentrations of this precursor peptide are also released into the blood. Whether the conversion to the hormonal plasma peptide occurs mainly in the heart, lung or plasma remains to be elucidated. Our experiments on the isolated perfused heart suggest that most of the plasma ANF is formed in cardiac tissue, similar to other hormones.

The release of ANF-like immunoreactive material in response to volume load is rapid. We find an increase of plasma concentration as soon as 1 min after stimulation. The exact mechanism involved in the mediation of release is unclear. As shown here, the stretch of atrial cardiocytes produced by volume expansion may induce secretion of the natriuretic and diuretic peptides. Whether, in addition, higher centres in the brain are involved via neuronal reflex arcs will be the subject of future experiments.

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1. Kisch, B. *Expl Med. Surg.* **14**, 99–112 (1956).
2. Palade, G. E. *Anat. Rec.* **139**, 262 (1961).
3. Marie, J. P., Guillemot, H. & Hatt, P. Y. *Path. Biol.* **24**, 549–554 (1976).
4. Sonnenberg, H., Veress, A. T., Borenstein, H. B. & de Bold, A. J. *Physiologist* **23**, 13 (1980).
5. Garcia, R., Cantin, M., Thibault, G. & Genest, J. *Experientia* **38**, 1071–1073 (1982).
6. Atlas, S. A. *et al. Nature* **309**, 717–719 (1984).
7. Flynn, T. G., de Bold, M. L. & de Bold, A. J. *Biochem. biophys. Res. Commun.* **117**, 859–865 (1983).
8. Geller, D. M. *et al. Biochem. biophys. Res. Commun.* **120**, 333–338 (1984).
9. Kangawa, K., Fukuda, A., Minamino, N. & Matsuo, H. *Biochem. biophys. Res. Commun.* **119**, 933–940 (1984).
10. Kangawa, K., Fukuda, A., Kubota, I., Hayashi, Y. & Matsuo, H. *Biochem. biophys. Res. Commun.* **121**, 585–591 (1984).
11. Maki, M. *et al. Nature* **309**, 722–724 (1984).
12. Oikawa, S. *et al. Nature* **309**, 724–726 (1984).
13. Seidah, N. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 2640–2544 (1984).
14. Seidmann, C. E. *et al. Science* **225**, 324–326 (1984).
15. Yamanaka, M. *et al. Nature* **309**, 719–722 (1984).
16. Langendorff, O. *Pflügers Arch. ges. Physiol.* **61**, 291–332 (1985).
17. Haddy, F. J. *Ann. intern. Med.* **93**, 781–784 (1983).
18. de Wardener, H. E. *Clin. Sci.* **21**, 249–258 (1961).
19. de Wardener, H. E. *Clin. Sci. molec. Med.* **53**, 1–8 (1977).
20. Sealey, J. E., Kirschmann, J. D. & Laragh, J. H. *J. clin. Invest.* **48**, 2210–2224 (1969).
21. Skowsky, W. R. & Fisher, D. F. *J. Lab. clin. Med.* **80**, 134–144 (1972).
22. Hunter, H. W. & Greenwood, F. C. *Nature* **194**, 495–496 (1984).

Homogeneous interferon-inducing 22K factor is related to endogenous pyrogen and interleukin-1

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***In vitro* stimulation of mononuclear cells from human peripheral blood with mitogens causes the release of factors (monokines and lymphokines) which possess distinct biological activities. One such factor, termed 22K, can induce production of human β-interferon (HuIFN-β) in cultured human fibroblasts, thereby rendering these cells resistant to virus infection[1–3]. Here we report the complete purification and partial sequencing (39 N-terminal amino acids) of this factor, whose relative molecular mass was estimated by SDS-polyacrylamide gel electrophoresis to be 17,000 (17K). In addition to an antiviral effect, the pure protein exhibits several other biological activities. Most significantly, intravenous (i.v.) injection of the factor in rabbits caused fever and granulopenia at doses of 0.1–1 μg per kg, effects which we attribute to a monokine called endogenous pyrogen (EP). *In vitro*, the protein was scored as positive in a LAF (lymphocyte-activating factor) assay at 0.1–1 ng ml^{-1}. LAF and EP are considered to be members of one family of monokines, called interleukin-1 (IL-1)[4]. For this reason, and also because the amino-acid sequence of the 22K factor is at least partially homologous to a complementary DNA-derived IL-1 sequence, we postulate that the 22K factor also belongs to the IL-1 family.**

Electrophoretically pure 22K was obtained from crude human lymphokine preparations by a four-step purification schedule summarized in Fig. 1 legend. The protein migrated on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as a single Coomassie blue-stained band with a relative molecular mass (M_r) of 17,000. A parallel unstained gel was sliced, each slice eluted and the eluates tested for antiviral activity. The antiviral activity coincided with the single stained band. We also determined the N-terminal amino-acid sequence of the protein using a gas-phase sequenator. Figure 2 shows the sequence of 39 N-terminal amino acids obtained from three runs, each using ~15 μg of protein. At most of the 39 positions, only a single residue was found in each of the three runs, indicating that the 22K-containing fractions obtained by fast protein liquid chromatography (FPLC) had a high degree of purity. At several positions two or three different residues were seen, suggesting that the factor is microheterogeneous. This microheterogeneity may reflect allelic polymorphism or the existence of a multi-gene family.

Although originally discovered by virtue of its IFN-β-like antiviral effect[2], 22K may have biological significance not related to the interferon system. In fact, the interferon-like antiviral effect of 22K is restricted in several ways: only certain diploid cell strains or continuous cell lines are sensitive and these may lose sensitivity on passage[3]; inhibition of the cytopathic effect is often incomplete, even with high doses of 22K; the reduction of virus yield generally does not exceed 3–10-fold; induction of HuIFN-β and its mRNA is detectable only when 22K is aided by cycloheximide or a combination of cycloheximide and actinomycin D[5].

Several experiments were done to determine whether 22K has biological activities previously assigned to 'factors' released by mononuclear cells; Table 1 summarizes the results. Pure 22K preparations did not inhibit the growth of cells on which they exerted an antiviral effect, nor did the factor show any activity in standard assay systems for lymphotoxin[6], interleukin-2 (IL-2)[7] and natural killer (NK) cell stimulation[8], while the LAF test for IL-1 (ref. 9) was positive. 22K preparations, including those consisting of electrophoretically homogeneous material, were able to replace crude lymphocyte supernatants as granulocyte-macrophage colony-stimulating factor (GM-CSF) in human bone marrow cultures[10], suggesting that 22K may cause the release of such factors from a subpopulation of bone marrow cells.

Strong inflammatory responses were seen when partially purified 22K preparations were injected intracutaneously in man, monkey or rabbit. Macroscopically, the reaction consisted of mild swelling and redness, maximal at 16 h post-injection and subsiding after 36–48 h. Microscopic examination of rabbit skin biopsies, taken 24 h after injection, revealed infiltration by polymorphonuclear cells, suggesting a direct or indirect chemotactic effect of the injected material. When fractions from gel filtration runs were tested in rabbits, the peak of skin reactivity coincided with that of antiviral activity. At this stage of purification, 22K may be presumed to still contain bioactive impurities, for example, IL-2, some of which may be responsible for the skin reactivity. However, on FPLC, skin reactivity also co-eluted with the electrophoretically pure antiviral factor.

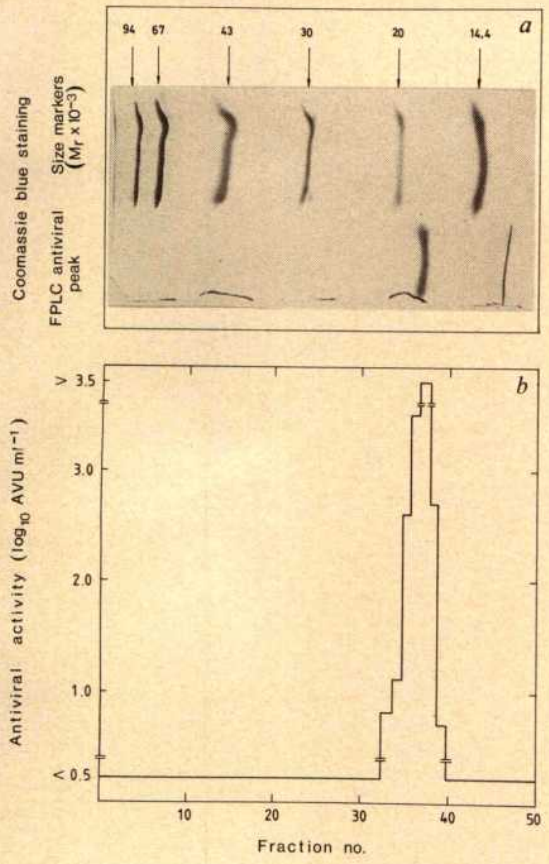


Fig. 1 SDS-PAGE of a pure preparation of 22K protein. Crude lymphokine/monokine preparations from Concanavalin A-induced mononuclear cell suspensions were concentrated and partially purified by adsorption to silicic acid and elution with 50% ethylene glycol containing phosphate-buffered saline (PBS) at *p*H 7.4 or with glycine buffer at *p*H 2.0 as described previously[2]. These eluates had an average specific activity of 10^4 AVU per mg protein; 1 AVU ml^{-1} is defined as the minimal concentration required to inhibit the cytopathic effect of vesicular stomatitis virus on sensitive human MG-63 cells, using a regular interferon microtitre assay system[2]. These preparations contained several lymphokines, including γ-interferon (IFN-γ) and IL-2 (results not shown). The second purification step consisted of adsorption of impurities to DEAE-Sephacel in 20 mM Tris-HCl buffer, *p*H 8.0. The DEAE-Sephacel-unadsorbed fraction (specific activity $10^{5.1}$ AVU mg^{-1}) was concentrated further and fractionated by gel filtration on Ultrogel AcA 54. Antiviral activity eluted in fractions corresponding to a M_r of 45,000 (fractions characterizable as IFN-γ) and 22,000 (fractions characterizable as IFN-β-like antiviral protein). The 22K fractions had a specific activity of $\geq 10^{6.5}$ AVU mg^{-1}; they were free of IFN-γ (M_r ~45,000), but were contaminated with IL-2 (M_r ~25,000). The 22K peak fraction from gel filtration was finally purified to homogeneity by FPLC using a Mono S cation-exchange column (Pharmacia Fine Chemicals); the activity was applied in 50 mM formiate buffer, *p*H 4.0. The Mono S column was then subjected to a linear NaCl gradient (0–0.65 M) at a flow rate of 1 ml min^{-1}. The antiviral activity invariably eluted as a single peak at 0.37 M NaCl. The specific activity was increased to $\geq 10^{7.0}$ AVU mg^{-1}. Fractions with high biological activity were pooled and analysed by SDS-PAGE on a linear 12% gel in reducing conditions. Coomassie blue-stained protein bands are shown in *a*. A parallel unstained gel was sliced and each slice was eluted with PBS; the eluates were stabilized with human serum albumin and dialysed against PBS to localize the antiviral activity (in *b*). For several experiments there was good quantitative agreement between the presence of the stained band and antiviral activity. Gels loaded with too little 22K failed to show the stained band and all fractions scored negatively. The protein content of antivirally active fractions, obtained at different purification steps, was measured by a fluorimetric assay, as described[2]. For fractions also containing Tris buffer protein, determinations were done using a Coomassie brilliant blue G-250 binding assay (Bio-Rad). In both cases bovine serum albumin was used as a standard.

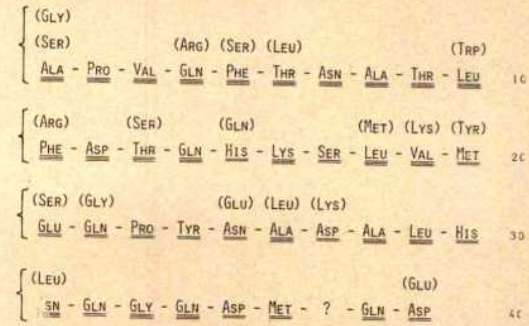


Fig. 2 N-terminal amino-acid sequence of the 22K factor. The number of lines beneath each residue indicates the number of runs in which the amino acid was observed. Brackets indicate detection of other residues.

Methods. Aliquots (~15 μg) of protein obtained from three FPLC runs (see Fig. 1 legend) were sequenced in a gas-phase sequenator[15] (Applied Biosystems, model 470A). The samples were not carboxymethylated before sequence analysis, so that cysteine residues could not be detected. The phenylthiohydantoin (PTH) amino acids obtained from the sequencer were identified by HPLC on an IBM cyanopropyl column (0.46 × 25 cm) using a separation procedure similar to that described by Hunkapiller and Hood[16]. The equipment used was the Waters Intellink system supplemented with a Perkin Elmer LC75 and a Pye Unicam LCUV detector set at 269 and 323 nm respectively.

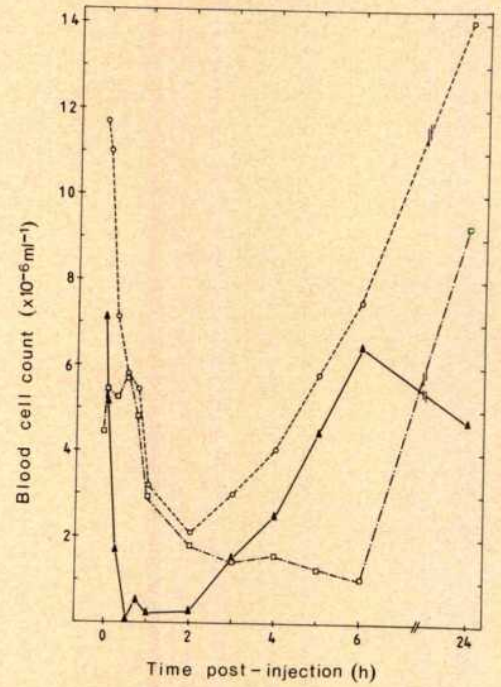


Fig. 3 Effect of i.v. injection of 22K protein on peripheral leukocyte counts in rabbits: ○ - - - ○, leukocytes (total); □ - - - □, lymphocytes; ▲—▲, granulocytes. The material injected was a FPLC-purified preparation (see Fig. 1 legend) containing ~1 μg of protein and $10^{4.3}$ AVU. Rabbits injected with saline or heat-treated (90 °C, 30 min) 22K factor did not show alterations in total or differential leukocyte counts.

Pure preparations of 22K were injected intravenously (i.v.) in rabbits to study the possible acute toxicity. Depending on the dose, increases in rectal temperature of up to 1.5 °C were noted; this effect was not due to contamination by bacterial endotoxins. Indeed, the injected 22K preparations lost their pyrogenicity after heat treatment (90 °C for 30 min), while the pyrogenicity of an *Escherichia coli* lipopolysaccharide preparation was heat-resistant. Pure preparations were also free of lipopolysaccharide as tested by the *Limulus* amoebocyte lysate assay (≤0.6 ng lipopolysaccharide in the highest injected dose, 3,000 antiviral units (AVU) per kg). Figure 3 shows that i.v. injection of pure 22K in rabbits caused a virtually immediate, but reversible, leukopenia. Granulocytes disappeared from the

Table 1 Biological properties of homogeneous 22K factor

Biological activity	Test system	Result (minimum effective dose or highest dose tested)
In vitro		
Antiviral effect	Inhibition of cytopathic effect in human diploid cells and MG-63 cells	Positive (1)*
CSF	Granulocyte-macrophage colony formation in human bone marrow cultures	Positive (1-3)*
IL-1	^{3}H-thymidine incorporation by human thymocytes	Positive (1)*
IL-2	^{3}H-thymidine incorporation by IL-2-dependent cytotoxic T-lymphocyte line	Negative (>500)*
NK-cell stimulation	Increase in natural cytotoxicity of human peripheral blood mononuclear cells against K-562 cells (^{51}Cr-release)	Negative (>100)*
Lymphotoxin	Cytolysis of mouse L-929 cells (subline α) pretreated with mitomycin C	Negative (>250)*
Cell growth	^{3}H-thymidine incorporation in log-phase cultures (human MG-63 and HEp-2)	Negative (>100)*
	Viable cell counts in log-phase cultures (human MG-63, HEp-2, U-937, HL-60 and diploid cells)	Negative (>100)*
In vivo		
Skin reactivity	Local inflammation after intracutaneous injection in rabbits	Positive (10,000)†
Pyrogenicity	0.5 °C Increase in rectal temperature after i.v. injection (3, 100 and 3,000 AVU per kg)	Positive (100 per kg)†
Leukopenia	Total and differential leukocyte counts after i.v. injection in rabbits (100, 1,000 and 10,000 AVU per kg)	Positive (1,000 per kg)

* Antiviral units (AVU) ml^{-1} as measured on MG-63 cells; † AVU.

circulating blood within 30 min after injection; lymphocyte counts decreased from 5,000 to a minimum of 2,000 cells per mm^3 in 2 h. Granulocytes reappeared from 2 h onwards and reached normal counts after 6 h; lymphocyte counts took 24 h to return to normal. The new cells were morphologically normal. Erythrocyte counts remained unaffected. The mechanism of this leukopenia is unclear, but present knowledge suggests a temporary adherence of the cells to the endothelia of the blood vessels and/or lymphoreticular organ system; however, other explanations are possible.

The *in vitro* antiviral effect of 22K occurred at doses of the order of 0.1-1 ng ml^{-1}, the systemic *in vivo* effect (leukopenia and pyrogenicity) at doses of 0.1-1 μg per kg. The molecule can thus be counted among the pharmacologically very active substances, such as lymphokines, interferons, neuropeptides, etc. Therefore, it seems reasonable to assume that 22K is a cytokine; the fact that it is released after mitogenic stimulation of leukocytes suggests that lymphocytes or monocytes are the producing cells and that 22K is generated in the body at sites where cellular immune reactions occur (for example, delayed-type hypersensitivity reactions, transplant rejection sites, etc.).

The strong pyrogenicity and haematological effects of 22K are both reminiscent of endotoxin-induced reactions[11], and suggest that 22K is related or identical to endogenous pyrogen. This monokine is considered to be related to IL-1 and to be involved in granulopoiesis[12], probably by stimulating production of CSFs. The conclusion that the 22K factor is essentially (an) endogenous pyrogen is therefore consistent with the fact that it has activity in a LAF assay and that it can replace CSF preparations in bone marrow cultures. The antiviral effect, which apparently is due to activation of the HuIFN-β system may, for reasons already mentioned, be of lesser physiological importance.

After submission of this paper, there have been reports on the isolation and sequencing of cDNA clones of human and murine IL-1 (refs 13, 14); these predict IL-1 gene products of M_r 31,000, which probably are 'precursor' proteins of the much smaller natural IL-1 products. The N-terminal amino-acid sequence of our 22K factor shows extensive homology with the middle part of the cDNA-derived human IL-1 precursor sequence. Alignment of the two sequences allows us to unambiguously define the position of the N-terminal amino acid of the biologically active human IL-1 in the precursor sequence determined by Auron *et al.*[13]. This provides further evidence that our 22K factor must be considered to be IL-1. Moreover, the fact that the homology was incomplete, together with the observation of multiple residues at several positions in the sequence, supports the notion that IL-1 comprises a microheterogeneous family of molecules.

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1. De Ley, M. *et al. Eur. J. Immun.* **10**, 877-883 (1980).
2. Van Damme, J. *et al. Eur. J. Immun.* **11**, 937-942 (1981).
3. Van Damme, J., Billiau, A., De Ley, M. & De Somer, P. *J. gen. Virol.* **64**, 1819-1822 (1983).
4. Dinarello, C. A. *Rev. infect. Dis.* **6**, 51-95 (1984).
5. Van Damme, J. *et al. J. gen. Virol.* (in the press).
6. Spofford, B. T., Daynes, R. A. & Granger, G. A. *J. Immun.* **112**, 2111-2116 (1974).
7. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. *J. Immun.* **120**, 2027-2032 (1978).
8. Claeys, H., Van Damme, J., De Ley, M., Vermylen, C. & Billiau, A. *Br. J. Haemat.* **50**, 85-94 (1982).
9. Maizel, A. L., Metha, S. R., Ford, R. J. & Lachman, L. B. *J. exp. Med.* **153**, 470-475 (1981).
10. Iscove, N. N., Senn, J. S., Till, J. E. & McCulloch, E. A. *Blood* **37**, 1-5 (1971).
11. Elin, R. J. & Wolff, S. M. *A. Rev. Med.* **27**, 127-141 (1976).
12. Kampschmidt, R. F. & Upchurch, H. F. *Proc. Soc. exp. Biol. Med.* **155**, 89-93 (1977).
13. Auron, P. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 7907-7911 (1984).
14. Lomedico, P. T. *et al. Nature* **312**, 458-462 (1984).
15. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. *J. biol. Chem.* **256**, 7990-7997 (1981).
16. Hunkapiller, M. W. & Hood, L. E. *Meth. Enzym.* **91**, 486-493 (1983).

A hapten-specific chimaeric IgE antibody with human physiological effector function

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Immunoglobulin E (IgE; see ref. 1) has a central role in allergic reactions although it rarely exceeds 5 μg ml^{-1} even in the serum of severely allergic individuals. Both mast cells and basophils possess receptors which bind the Fc portion of IgE with high affinity; crosslinking of membrane-bound IgE by allergen results in degranulation of the cell and release of a variety of pharmacologically active mediators including histamine. Myeloma IgE

has been successfully used to block the skin sensitizing activity of allergic sera[2]; however, human myeloma IgE is clearly in limited supply. The emergence of techniques allowing the stable introduction of immunoglobulin gene DNA into myeloma cells[3–5] has allowed us to construct a mouse cell line that secretes a chimaeric IgE, λ1 antibody whose heavy chain is composed of a human C_ε constant region fused to a mouse variable (V_H) region. This chimaeric IgE is specific for the hapten 4-hydroxy-3-nitrophenacetyl (NP) and can, when crosslinked by antigen, trigger the degranulation of human basophils. When not crosslinked, however, the chimaeric IgE can prevent the passive sensitization of these cells by sera from allergic subjects.

The mouse plasmacytoma J558L secretes λ1 light chains but does not produce an immunoglobulin heavy chain[3]. The strategy used in the production of the recombinant IgE antibody was to introduce a plasmid that encodes a complete immunoglobulin ε heavy chain into J558L and thus direct the production of an IgE, λ1 antibody. The plasmid used for this purpose, pSV-$V_{NP}H\varepsilon$ (see Fig. 1), consists of an immunoglobulin ε heavy-chain gene cloned into the vector pSV2gpt (ref. 6). The heavy chain consists of a mouse variable region and a human ε constant region. This heavy chain, in combination with the λ1 light chain expressed by the J558L myeloma, should produce an antibody specific for the hapten NP. In plasmid pSV-$V_{NP}H\varepsilon$, the exons encoding the variable (V_H) and constant (C_H) regions of the heavy chain are separated by only 300 base pairs of intron; this contrasts with the normal *in vivo* situation where the two sets of exons are separated by an intron of ~8 kilobases (kb). The transcription enhancer element[4,7,8], which normally lies between V_H and C_H, is located upstream of the V_H exons in pSV-$V_{NP}H\varepsilon$; we have shown previously that the enhancer is active on the V_{NP} promoter at this position[4].

pSV-$V_{NP}H\varepsilon$ was introduced into J558L cells by spheroplast fusion, and stably transfected clones were selected as described previously[9]. Transfectants were obtained with a frequency of between 10^{-3} and 10^{-4} in different experiments. Culture supernatants of these transfectants were assayed for production of λ1-bearing anti-NP antibody by radioimmunoassay; between 50 and 80% of the clones were positive. Antibody was purified from culture supernatants of several transfected clones by affinity chromatography on a hapten–Sepharose column, with a yield of ~2 mg of antibody per litre.

Binding inhibition assays were used to demonstrate that the purified antibody displayed human ε antigenic determinants (Fig. 2). The affinity-purified chimaeric antibody competes with the binding of radiolabelled human myeloma IgE to both monoclonal (Fig. 2*a*) and polyspecific (Fig. 2*b*) anti-ε antisera. Furthermore, binding of radiolabelled myeloma IgE to the anti-ε antiserum was inhibited completely (Fig. 2*b*), indicating that the chimaeric antibody displays all the ε antigenic determinants recognized by this polyclonal antiserum. The chimaeric antibody competed with the binding of the radiolabelled myeloma IgE to the anti-ε antisera better than did the unlabelled myeloma

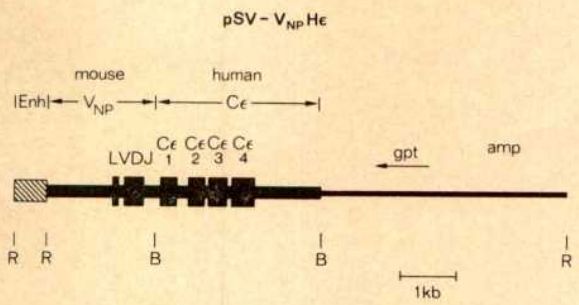


Fig. 1 Structure of plasmid pSV-$V_{NP}H\varepsilon$. Immunoglobulin DNA is represented by thick lines, pSV2gpt vector sequences by thin lines and the mouse C_H locus transcription enhancer element by a hatched box. Restriction endonuclease cleavage sites: R, *Eco*RI; B, *Bam*HI.

Methods. The *Eco*RI-*Bam*HI fragment of plasmid pSV-Vμ1 (ref. 4), which includes the V_H exons, was cloned between the *Eco*RI and *Bam*HI sites of plasmid pSV2gpt[6] to yield pSV-V_{NP}. The *Bam*HI fragment of phage λε1.2 (ref. 14), which includes exons C_ε1 to C_ε4 of the human ε gene, was cloned into pSV-V_{NP}, and plasmid pSV-$V_{NP}H\varepsilon$ completed by including in its unique *Eco*RI site the *Xba*I-*Eco*RI enhancer-containing fragment of the mouse heavy-chain locus.

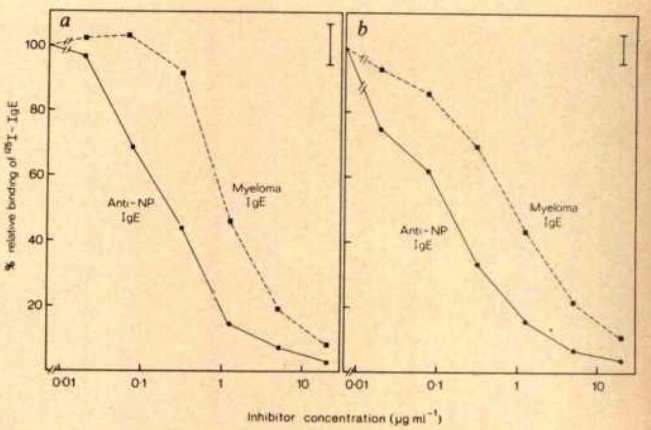


Fig. 2 Serological analysis of chimaeric IgE by binding inhibition assays. Assays were performed in which the binding of a radioiodinated human myeloma IgE to either a monoclonal (*a*) or polyclonal (*b*) anti-human ε antiserum was inhibited by various concentrations of unlabelled chimaeric IgE (●) or unlabelled myeloma IgE (■).

Methods. Wells of a Dynatec microtitre plate were coated with a solution containing either 1 μg ml^{-1} of a monoclonal mouse anti-human ε antibody (antibody RB6-2; given by M. D. Cooper) or 3 μg ml^{-1} of a polyclonal sheep anti-human ε antiserum (Seward Laboratory). After blocking of unreacted sites with bovine serum albumin, a human myeloma IgE (Serotec) that had been radiolabelled with ^{125}I was incubated in the wells in the presence of different concentrations of either chimaeric IgE or unlabelled myeloma IgE itself.

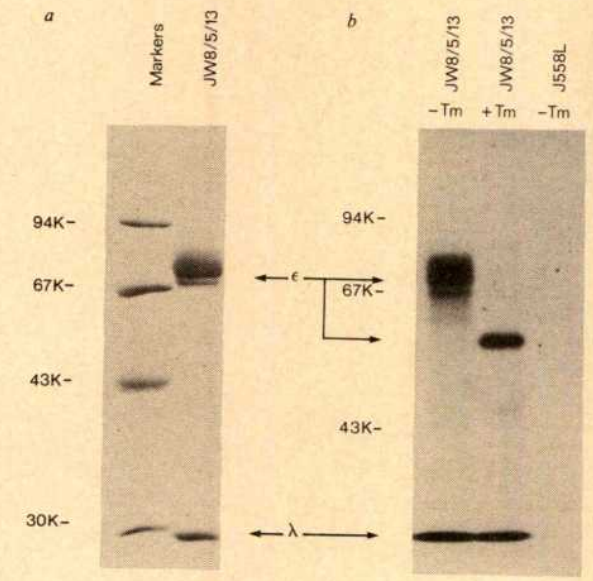


Fig. 3 Analysis of chimaeric IgE by SDS-polyacrylamide gel electrophoresis. *a*, Purified antibody (30 μg) from a cloned pSV-$V_{NP}H\varepsilon$ transfectant of J558L (JW8/5/13) was boiled in the presence of 2-mercaptoethanol, subjected to electrophoresis through a 9% gel and stained with Coomassie blue. *b*, Biosynthetically labelled antibody from either J558L or the transfectant JW8/5/13 was purified on NIP-Sepharose columns and analysed on a 7% gel after reduction. Samples purified from cells labelled in the presence of tunicamycin are marked +Tm. The positions of relative molecular mass markers are indicated (94K = relative molecular mass 94,000). Biosynthetic labelling with [L-^{35}S]methionine and purification of both labelled and unlabelled samples on hapten–Sepharose columns were performed as described previously[9].

Table 1 Histamine release triggered from human basophils passively sensitized with chimaeric IgE

Antigen	% Histamine release (±s.e.m.)
—	6.4 ± 0.8
10 μg ml^{-1} NIP-BSA	18.4 ± 2.4
1 μg ml^{-1} NIP-BSA	36.0 ± 5.6
0.1 μg ml^{-1} NIP-BSA	31.2 ± 12.0
0.01 μg ml^{-1} NIP-BSA	26.4 ± 2.4
0.001 μg ml^{-1} NIP-BSA	7.2 ± 1.6
0.0001 μg ml^{-1} NIP-BSA	5.2 ± 1.2
20 μg ml^{-1} Antigen P1	4.5 ± 0*

Mononuclear cells were prepared from peripheral blood by sedimentation through dextran-EDTA[11]; these preparations contained 1-2% basophils. Cells were incubated in duplicate at 37 °C for 2 h in complete Tyrode's buffer[12] with 1.5 μg ml^{-1} chimaeric anti-NP IgE. Cells were then centrifuged and resuspended in complete Tyrode's buffer containing the indicated concentrations of $(NIP)_{30}$-BSA. After 15 min, the histamine released into the supernatant was extracted and assayed fluorimetrically[11]. The maximum histamine release that could be obtained by incubating the cells at 100 °C was 62.5 ng ml^{-1}. The results are expressed as percentage histamine release: (antigen-induced histamine release [ng ml^{-1}]) × 100/maximum histamine release obtainable (ng ml^{-1}). The mean background percentage release obtained in repeated experiments was 6.0 ± 2.5. Both NIP-BSA (1.0 μg ml^{-1}) and antigen P1 (20 μg ml^{-1}) alone repeatedly failed to induce histamine release above background levels.

* Data obtained in a separate experiment using cells from the same donor.

Table 2 Blocking of histamine release by preincubation with chimaeric IgE

Concentration (μg ml^{-1}) of chimaeric anti-NP IgE in preincubation	Serum from allergic subject	Antigen P1	% Histamine release (±s.e.m.)
—	+	+	20.5 ± 2.7
0.01	+	+	16.9 ± 2.4
0.1	+	+	7.2 ± 0
1	+	+	8.2 ± 1.0
10	+	+	7.2 ± 1.0
10	−	−	8.4 ± 1.2
—	−	+	7.5 ± 1.7

Mononuclear cells were prepared as described in Table 1. A 2-h preincubation with different concentrations of chimaeric anti-NP IgE was followed by a 2-h incubation with allergic serum (diluted 1:30 in complete Tyrode's), prior to a 15-min exposure to antigen P1 (20 μg ml^{-1}). The allergic serum used had a total IgE level of 4,800 IU ml^{-1}, and 1,600 BA units ml^{-1} of IgE specific for *D. pteronyssinus* antigen P1 (ref. 13). The maximum histamine release obtained by incubating the cells used in this experiment at 100 °C was 10.4 ng ml^{-1}.

IgE itself, probably because the purified chimaeric antibody is essentially homogeneous (see Fig. 3), whereas the commercial sample of myeloma IgE is not.

The structure of the chimaeric protein was investigated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Examination of reduced anti-NP IgE reveals a band co-migrating with mouse λ1 light chains and a diffuse heavy-chain band of relative molecular mass (M_r) ~72,000 (72K) (Fig. 3*a*); this is considerably larger than the M_r of the ε polypeptide chain as predicted from the DNA sequence. However, this discrepancy is resolved by SDS-PAGE of biosynthetically labelled anti-NP IgE secreted by cells labelled in the presence of tunicamycin (Fig. 3*b*). Incubation with this glycosylation inhibitor reduces the apparent relative molecular mass of the secreted anti-NP IgE from 72K to the predicted value of 62K. Thus the chimaeric IgE, like IgE from human myeloma[10], is heavily glycosylated. The electrophoretic mobility of unreduced anti-NP IgE in SDS-polyacrylamide gels (not shown) is consistent with it having an $\varepsilon_2\lambda_2$ structure.

Two types of assay were performed to determine whether the chimaeric anti-NP IgE antibody exhibits the physiological effector functions of authentic human IgE. In one type of assay, we tested the ability of the antibody to trigger histamine release from human basophils. A preparation of mononuclear cells from peripheral blood containing 1-2% basophils was passively sensitized with the chimaeric antibody before incubation with hapten (5-iodo-4-hydroxy-3-nitrophenacetyl caproate, NIP) coupled to bovine serum albumin (NIP-BSA). The results (Table 1) indicate that, after preincubation of basophils with the chimaeric IgE, NIP-BSA is able to trigger a dose-dependent release of histamine. A heterologous antigen, antigen P1 of the house dust mite *Dermatophagoides pteronyssinus*, failed to induce histamine release. Similarly, only background levels of histamine release were obtained with either the chimaeric antibody or NIP-BSA alone. These results indicate that the anti-NP IgE, like authentic human IgE, will not cause degranulation by itself, but will trigger histamine release following crosslinking with antigen.

In a second assay, we demonstrated that incubation with anti-NP IgE could also block the subsequent passive sensitization of basophils by atopic sera containing high IgE levels. Table 2 shows that house dust mite antigen P1 can induce the release of histamine from basophils preincubated with serum containing anti-P1 IgE antibody. However, this same serum failed to effect histamine release when the cells had been incubated previously with the chimaeric antibody at concentrations >0.1 μg ml^{-1}.

The results described here demonstrate that transfection of DNA into mouse myeloma cells is an effective way of producing large amounts of chimaeric antibodies in which mouse *V* regions provide antigen-binding specificity and human C_H regions provide human effector functions. The known antigen-binding specificity of such an antibody makes its purification extremely simple. Production of a chimaeric IgE in this way has proved particularly attractive as no monoclonal human IgE of known antigen specificity was previously available. The chimaeric antibody may, therefore, prove useful in routine clinical assays. We have demonstrated that this monoclonal IgE is able to block the release of histamine from human basophils which can be triggered *in vitro* by sera from allergic subjects. It will clearly be important to discover whether analogous blocking can be achieved *in vivo* using skin sensitization assays.

We thank S. Morrison for the J558L myeloma, M. D. Cooper for the monoclonal anti-ε antibody and R. R. Coombs for critical reading of the manuscript.

Note added in proof: Morrison *et al.*[15] and Boulianne *et al.*[16] have recently described the construction of chimaeric IgG antibodies.

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1. Möller, G. (ed.) *Immun. Rev.* **41** (1978).
2. Stanworth, D. R., Humphrey, J. H., Bennich, H. & Johansson, S. G. O. *Lancet* **ii**, 330-332 (1967).
3. Oi, V. T., Morrison, S. L., Herzenberg, L. A. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 825-829 (1983).
4. Neuberger, M. S. *EMBO J.* **2**, 1373-1378 (1983).
5. Ochi, T. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 6351-6355 (1983).
6. Mulligan, R. C. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2072-2076 (1983).
7. Banerji, J., Olson, L. A. & Schaffner, W. *Cell* **33**, 729-740 (1983).
8. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717-728 (1983).
9. Neuberger, M. S., Williams, G. T. & Fox, R. O. *Nature* **312**, 604-608 (1984).
10. Johansson, S. G. O. & Bennich, H. *Immunology* **13**, 381 (1967).
11. Lichtenstein, L. M. & Osler, A. G. *J. exp. Med.* **120**, 507-530 (1964).
12. Ennis, M. *Ag. Actions* **12**, 60-63 (1982).
13. Chapman, M. D. & Platts-Mills, T. A. E. *J. Immun.* **125**, 587-592 (1980).
14. Flanagan, J. G. & Rabbitts, T. H. *EMBO J.* **1**, 655-660 (1982).
15. Morrison, S. L., Johnson, M. J., Herzenberg, L. A. & Oi, V. T. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6851-6855 (1984).
16. Boulianne, G. L., Hozumi, N. & Shulman, M. J. *Nature* **312**, 643-646 (1984).

The gene encoding the T-cell receptor α-chain maps close to the *Np-2* locus on mouse chromosome 14

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Serological and molecular genetic analyses of T-cell clones have shown that the T-cell antigen receptor[1] apparently comprises two glycosylated, disulphide-linked polypeptide chains (α and β), both of which span the cell membrane. Cloning of the genes encoding the two chains from mouse and human DNA has shown that the α- and β-chains are composed of variable (V) and conserved (C) regions in agreement with peptide mapping data[2–11]. Gene segments encoding variable and conserved domains of the β-chain have been identified and undergo rearrangements during T-cell differentiation. The genes encoding the α-chain, so far described at the level of complementary DNA clones, also identify DNA rearrangements. Thus, the genes encoding the T-cell receptor show the same structure and dynamic behaviour as immunoglobulin genes, indicating that the two gene families belong to the same supergene family[5,12,13]; this evolutionary relationship is supported by the fact that the genes encoding the β-chain of the T-cell receptor are closely linked to immunoglobulin κ light-chain genes on chromosome 6 in mouse[14]. In man, however, the β genes map to chromosome 7 (ref. 14) whereas the κ-chain genes are located on chromosome 2, indicating that linkage between the two gene families is not needed for proper expression. Here we describe genomic clones encoding the constant portion of the T-cell receptor α-chain and map the gene to chromosome 14 in mouse, close to the gene for purine nucleoside phosphorylase (*Np*-2) which, in man, has been associated with T-cell immunodeficiencies.

The strategy used to clone the mouse α-chain gene involved the synthesis of a 33-nucleotide long oligomer based on published sequences[9,10] of α-chain cDNA clones. The oligomer, corresponding to the 5′ end of the constant region, was used to screen a BALB/c mouse thymocyte cDNA library constructed in the phage vector λgtII and consisting of ~10^6 independent clones. About 30 positive clones were scored, one of which was picked, rescreened, grown up and subcloned in the plasmid vector pUC9. This clone (T1.2), containing a cDNA insert of 1,100 base pairs (bp), was then characterized with five different restriction enzymes; the map obtained (Fig. 1) agrees completely with those published for α-chain cDNA clones[9,10]. The comparison also indicated that clone T1.2 contains *V*, joining (*J*), and 3′-untranslated region sequences. DNA sequence determination of a portion of the variable and constant region of T1.2 further confirmed its identity as an α-chain cDNA clone (E. Karjalainen, personal communication).

The *Nco*I fragment (probe 1 in Fig. 1) was then used to screen a mouse cosmid library constructed with BDFL 1.1 DNA. BDFL 1.1 is a cytotoxic T-cell clone obtained from a (C57BL/6J × DBA/2J)F_1 mouse and is specific for fluorescein in association with H-2D^d (W. Haas, unpublished data); 28 positive clones were detected initially, 5 of which were selected finally after screening with probe 2 (see Fig. 1). Mapping of these 5 cosmid clones with 14 different restriction enzymes showed that they all contain overlapping DNA sequences. Four enzymes, however, detected four polymorphic restriction sites; these sites defined two groups of overlapping cosmid clones, presumably representing the two constant-region alleles present in BDFL 1.1. Figure 2 shows one clone for each group of cosmid clones representing the two alleles; both of these clones contain the gene for the constant region of the α-chain, as shown by

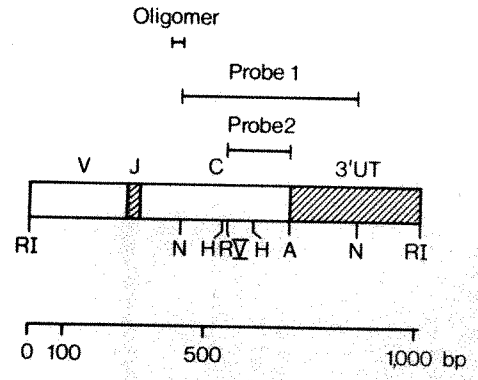


Fig. 1 Restriction enzyme map of the insert of cDNA clone T1.2. Restriction enzymes are: A, *Ava*II; H, *Hin*dIII; RI, *Eco*RI; RV, *Eco*RV. The *Hin*dIII site in the pUC9 vector molecule is located to the left. The locations of the oligomer and probes 1 and 2 are indicated.

Methods. The cDNA library was constructed with BALB/c thymocyte mRNA in λgtII as described elsewhere[28] and screened with a 33-nucleotide long oligomer corresponding to amino-acid residues 141–151 (ref. 9) of the constant region of the T-cell receptor α-chain. The oligonucleotide was synthesized on controlled-pore glass as support, using phosphite chemistry[29]. Hybridization with T4 kinase-labelled oligomer was done in 50% formamide, 5 × SSC, 0.1% SDS, 5 × Denhardt's solution, 10% dextran sulphate, 50 mM sodium phosphate (*p*H 7), 1 mM sodium pyrophosphate, 100 μM ATP, 50 μg ml^{-1} *Escherichia coli* DNA and 50 μg ml^{-1} poly (A) at 42 °C overnight. Filters were washed in 6 × SSC, 0.1% SDS twice for 20 min each at 42 °C and exposed to X-ray film. Of 30 positive clones, one was picked, rescreened, grown up and the *Eco*RI insert subcloned into pUC9 using standard procedures[30]. The clone (T1.2) was mapped by single and double digestions using the restriction enzymes indicated.

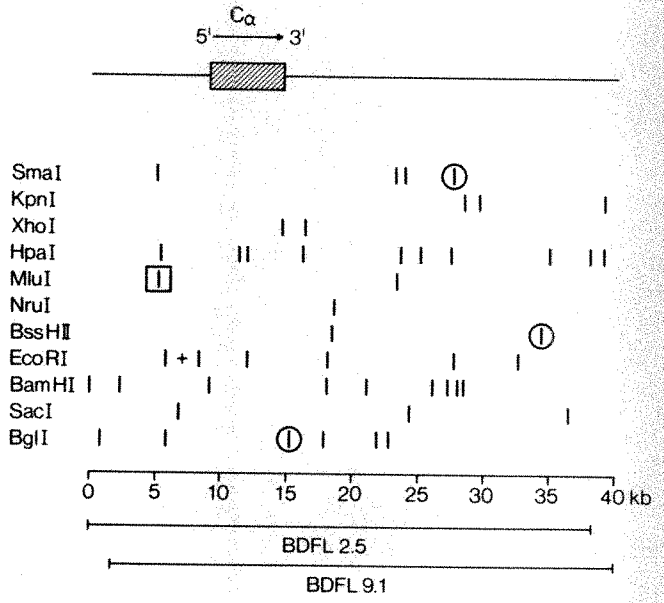


Fig. 2 Molecular map of a 40-kb region of DNA around the constant-region gene of the T-cell receptor α-chain. The region is defined by the two cosmid clones BDFL 2.5 and BDFL 9.1 derived, respectively, from the DBA/2 and the C57BL/6 chromosome of the cytotoxic T-cell clone BDFL 1.1 (see text). Restriction enzymes sites are indicated by vertical bars; those specific for the C57BL/6 allele are circled and the DBA/2-specific site is indicated by a square. No sites were found for *Sal*I, *Cla*I or *Sac*II. + Indicates that one additional *Eco*RI site is present in the segment marked. The extent of the C_α gene is only approximate and was determined by Southern blot hybridization of restriction fragments from the cosmid clones with the probes described in Fig. 1 legend. The cosmid library of BDFL 1.1 DNA was constructed using the vector pNNL and screened with oligo-labelled[31] probe 1 (Fig. 1) as described elsewhere[32]. Cosmid clones were analysed by restriction enzyme digestion and hybridization as described[32,33].

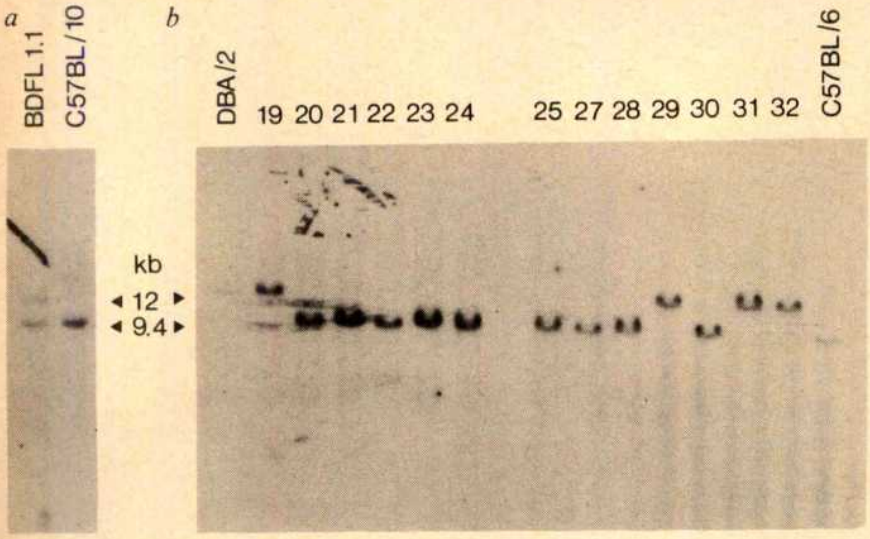


Fig. 3 Southern blot analysis of *Bgl*I-digested mouse DNA with a C_α probe. *a*, DNA from C57BL/10 mouse liver and the BDFL 1.1 cell clone. *b*, DNA from DBA/2 and C57BL/6 mouse livers and 13 recombinant inbred strains of the BXD series (see Table 1). DNA (5–10 μg of each sample) was digested to completion and separated on a 0.6% agarose gel, transferred to nitrocellulose filters and hybridized with oligo-labelled[31] probe 2 (Fig. 1) as described previously[33]. BXD DNAs were obtained from the Jackson Laboratory.

hybridization with the cDNA probes 1 and 2 and the synthetic oligomer. Hybridization of distinct restriction fragments to the oligomer (5′ probe) and the *Nco*I fragment (3′ probe) defined the 5′ to 3′ orientation of the constant-region gene (Fig. 2). As DNA rearrangements, resulting from *V–J* joining, are presumed to occur upstream of the constant-region gene, we are uncertain as to whether the polymorphism revealed by the *Mlu*I site is a result of a possible *V–J* joining on one or both chromosomes.

To confirm that the polymorphic restriction sites on cosmid clones 9.1 and 2.5 do indeed characterize the α-chain alleles derived from DBA/2 and C57BL/6 DNA, we performed Southern blotting experiments using probe 2. As expected from the cosmid clones, two *Bgl*I fragments of 12 and 9.4 kilobases (kb) were identified in BDFL 1.1 DNA (Fig. 3*a*). These fragments were identical in size to the 12-kb *Bgl*I fragment detected in DBA/2 DNA and the 9.4-kb fragment found in C57BL/10 or C57BL/6 (Fig. 3*b*) DNA, indicating that cosmid clone 2.5 corresponds to the DBA/2 allele whereas cosmid clone 9.1 represents the C57BL/6 allele.

The restriction site polymorphism revealed between DBA/2 and C57BL/6 DNA immediately suggested the use of recombinant inbred strains of mice to determine the location of the α-chain gene in the genome of the mouse. Recombinant inbred strains are derived by crossing two genetically distinct progenitor strains and establishing multiple independent strains from the F_2 generation by continuous brother-sister inbreeding[15]. The chromosomes of recombinant inbred strains consist of alternating segments derived from the two progenitor strains, the boundaries of which are determined by chance fixation of crossovers during the inbreeding process. Once inbred, the recombinant strains are typed for genetic loci that discriminate between the progenitors to obtain a strain distribution pattern characteristic for each locus. As closely linked genes will tend to become fixed in the same combination as they entered the cross, recombinant inbred strains are useful for detecting linkages and for estimating recombination frequencies.

Table 1 Strain distribution pattern of the *Bgl*I polymorphism of the α gene

Strain	Restriction fragment	Strain	Restriction fragment
BDFL 1.1	12 kb(D), 9.4 kb(B)	BXD 16	D
DBA/2	D	BXD 18	D
C57BL/10	B	BXD 19	D
C57BL/6	B	BXD 20	B
BXD 1	B	BXD 21	B
BXD 2	D	BXD 22	B
BXD 5	B	BXD 23	B
BXD 6	D	BXD 24	B
BXD 8	D	BXD 25	B
BXD 9	D	BXD 27	B
BXD 11	D	BXD 28	B
BXD 12	D	BXD 29	D
BXD 13	B	BXD 30	B
BXD 14	D	BXD 31	D
BXD 15	D	BXD 32	D

*Bgl*I fragments detected with probe 2 (Fig. 1) in the various recombinant inbred strains listed were either 12 kb (derived from DBA/2, D allele) or 9.4 kb (derived from C57BL/6, B allele) long. The heterozygous BDFL 1.1 cell clone (C57BL/6 × DBA/2) contained a 12- and a 9.4-kb *Bgl*I fragment (compare with Fig. 3).

The BXD series of recombinant inbred strains consists of 26 strains derived from crossing C57BL/6J with DBA/2J mice[16]. Over 150 loci have now been typed. We tested DNA of the 26 strains after *Bgl*I digestion with probe 2 and obtained the strain distribution pattern for the α-chain gene shown in Fig. 3*b* and Table 1. This pattern shows perfect concordance with the strain distribution pattern of the *Np-2* locus, indicating with 95% certainty that the α-chain gene maps within 3.3 centimorgans of *Np-2* (ref. 17). The probability of finding complete concordance for two unlinked loci by chance alone is $<1\times10^{-7}$ in the BXD series.

The *Np-2* locus is closely linked to the *Np-1* locus[18] on chromosome 14 in the mouse[19]. Thus, neither the α- nor the β-chain gene for the T-cell receptor is located on chromosome 12 in the mouse, which carries the immunoglobulin heavy-chain genes. The finding of immunoglobulin allotype-linked idiotypes on T cells therefore remains unexplained[20,21].

In C57BL mice, *Np-2* controls an erythrocyte-specific isozyme of purine nucleoside phosphorylase, an enzyme important for the catabolism of purine nucleotides[22]. The common form of this enzyme found in all inbred strains studied, including C57BL, is encoded by the *Np-1* locus. Interestingly, in man, deficiencies of purine nucleoside phosphorylase have been shown to be associated with severe T-cell immunodeficiencies[23]. It has been proposed that elevated levels of dGTP accumulating in the absence of the enzyme are toxic to T lymphocytes[24]. Given the apparent close linkage between the genes for the T-cell receptor α-chain and nucleoside phosphorylase in the mouse, it is likely that the human α-chain gene is also linked to the nucleoside phosphorylase locus[25]. If this is the case, at least some of the phosphorylase-linked T-cell deficiencies in man might be the result of genetic defects affecting both loci; this can be tested as the location of the phosphorylase gene in man is known (incidentally also on chromosome 14; ref. 26) and the gene has been cloned[27].

Complete concordance between the strain distribution patterns for the *Np-2* locus and the α-chain gene is also observed

in the BXH (12 strains) and CXB (7 strains) series of recombinant inbred strains. Furthermore, the two *H-8* congeneic mouse strains, B6.C-*H-8*^{c}/By and B10.D2 (57N)/Sn, contain the BALB/c and DBA/2 alleles, respectively, at both the *Np-2* and α-chain gene loci. We propose *Tcra* as a symbol for the T-cell receptor α-chain gene locus.

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1. Robertson, M. *Nature* **312**, 16-17 (1984).
2. Acuto, O. *et al. Cell* **34**, 717-726 (1983).
3. Kappler, J. *et al. Cell* **35**, 295-302 (1983).
4. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308**, 149-153 (1984).
5. Yanagi, Y. *et al. Nature* **308**, 145-149 (1984).
6. Siu, G. *et al. Cell* **37**, 393-401 (1984).
7. Malissen, M. *et al. Cell* **37**, 1101-1110 (1984).
8. Gascoigne, N. R. J., Chien, Y., Becker, D. M., Kavaler, J. & Davis, M. M. *Nature* **310**, 387-391 (1984).
9. Chien, Y. *et al. Nature* **312**, 31-35 (1984).
10. Saito, H. *et al. Nature* **312**, 36-40 (1984).
11. Hannum, C. H., Kappler, J. W., Trowbridge, I. S., Marrack, P. & Freed, J. *Nature* **312**, 65-67 (1984).
12. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153-158 (1984).
13. Siu, G. *et al. Nature* **311**, 344-350 (1984).
14. Caccia, N. *et al. Cell* **37**, 1090-1099 (1984).
15. Bailey, D. W. in *The Mouse in Biomedical Research* Vol. 1 (eds Foster, H. L., Small, J. D. & Fox, J. G.) 223-239 (Academic, New York, 1981).
16. Taylor, B. A. in *Genetic Variants and Strains of the Laboratory Mouse* (ed. Green, M. C.) 397-407 (Fischer, Stuttgart, 1979).
17. Taylor, B. A. in *Origins of inbred Mice* (ed. Morse, H. C. III) 423-428 (Academic, New York, 1978).
18. Taylor, B. A. *Mouse News Lett.* **65**, 28 (1981).
19. Womack, J. E., Davisson, M. T., Eicher, E. M. & Kendall, D. A. *Biochem. Genet.* **15**, 347-355 (1977).
20. Binz, H., Wigzell, H. & Bazin, H. *Nature* **264**, 639-642 (1976).
21. Rajewski, K. & Eichmann, K. *Contemp. Topics Immunobiol.* **7**, 69-112 (1977).
22. Bremner, T. A., Premkumar-Reddy, E., Nayor, K. & Kouri, R. E. *Biochem. Genet.* **16**, 1143-1151 (1978).
23. Giblett, E. L., Ammann, A. J., Wara, D. W., Sandman, R. & Diamond, L. K. *Lancet i*, 1010-1013 (1975).
24. *Gudas, L. J., Ullman, B., Cohen, A. & Martin, D. W. Jr Cell* **14**, 531-538 (1978).
25. Nadeau, J. H. & Taylor, B. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 814-818 (1984).
26. George, D. L. & Francke, U. *Science* **194**, 851-852 (1976).
27. Williams, S. R., Goddard, J. M. & Martin, D. W. Jr *Nucleic Acids Res.* **12**, 5779-5787 (1984).
28. Goridis, C. *et al. EMBO J.* (in the press).
29. Kiefer, H. R. & Bannwarth, W. in *Immunological Methods* Vol. 3 (eds Lefkovits, I. & Pernis, B.) (Academic, New York, in the press).
30. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1983).
31. Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **137**, 266-267 (1984).
32. Steinmetz, M., Stephan, D., Dastoornikoo, G. R., Gibb, E. & Romaniuk, R. in *Immunological Methods* Vol. 3 (eds Lefkovits, I. & Pernis, B.) (Academic, New York, in the press).
33. Steinmetz, M. *et al. Nature* **300**, 35-42 (1982).

The human T-cell receptor α-chain gene maps to chromosome 14

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The T-cell receptor for antigen has been identified as a disulphide-linked heterodimeric glycoprotein of relative molecular mass (M_r) 90,000 comprising an α- and a β-chain[1-3]. The availability of complementary DNA clones encoding mouse[4] and human[5] β-chains has allowed a detailed characterization of the genomic organization of the β-chain gene family and has revealed that functional β-chain genes in T cells are generated from recombination events involving variable (*V*), diversity (*D*), joining (*J*) and constant (*C*) gene segments[6,7]. Recently, cDNA clones encoding mouse[8,9] and human[10] α-chains have been described; the sequences of these clones have indicated that functional α-chain genes are also generated from multiple gene segments. It is possible that chromosomal translocations involving T-cell receptor α- and β-chain genes have a role in T-cell neoplasms in much the same way as translocations involving immunoglobulin genes are associated with oncogenic transformation in B cells[11]. In the latter case, the chromosomal localization of the immunoglobulin genes provided one of the first indications of the involvement of such translocations in oncogenic transformation. The chromosomal assignment of the α- and β-chain genes may, therefore, provide equally important clues for T-cell neoplastic transformation. The chromosomal location of the mouse and human β-chain gene family has been determined: the murine gene lies on chromosome 6 (refs 12, 13) whereas the human gene is located on chromosome 7 (refs 13, 14). Here we use a cDNA clone encoding the human α-chain to map the corresponding gene to chromosome 14.

The α-chain cDNA clone used in this study was isolated from a $\phi\lambda$gt10 library, constructed from messenger RNA isolated from the human T-cell line Jurkat[15], by cross-hybridization with a homologous mouse probe[8]. The sequence of the insert corresponds exactly to that of a previously published α-chain cDNA clone isolated from HPB-MLT[10] and comprises about three-quarters of the constant region plus almost the entire 3′-untranslated region of the human α-chain (M.K.L.C., M.J.O., G.T. and S.T., manuscript in preparation).

The insert (~900 base pairs, bp) from the $\phi\lambda$gt10 clone was subcloned into pBR322 and the resulting plasmid (pJ6α2) used to screen human and mouse DNA, and a panel of mouse/human somatic cell hybrid DNAs using Southern hybridization analysis. Human DNA digested with *Hin*dIII and hybridized with the pJ6α2 insert shows two bands of 4.2 and 2.9 kilobases (kb)

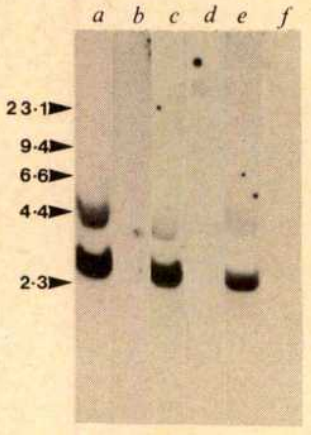


Fig. 1 Southern hybridization analysis of mouse/human somatic cell hybrid DNA. DNA of high M_r was prepared according to Maniatis *et al.*[34] and 20 μg was digested with *Hin*dIII and electrophoresed through 0.8% agarose, blotted onto nitrocellulose and hybridized with oligo-labelled[35] insert from pJ6α2. Hybridization was carried out at 65 °C in 3×SSC. The filters were washed at 65 °C in 0.2×SSC. The sizes of the DNA fragments were determined by reference to the mobilities of $\phi\lambda$*Hin*dIII markers. *a*, Human fibroblast (Bu) DNA; *b*, mouse L-cell (1R) DNA; *c*, hybrid F4SC13 C19 DNA; *d*, hybrid HORL411B6P DNA; *e*, hybrid FIR5R3 DNA; *f*, hybrid DT1.2R DNA.

Table 1 Segregation of a human α-chain gene in somatic cell hybrids

Hybrid (ref.)	Human chromosomal constitution	Presence of human-specific bands*
DUR4.3 (25)	3, 10, 11, 12, 13, 14, 15, 17, 18, 20, 21, 22, X	+
CTP34B4 (26)	1, 2, 3, 5, 6, 7, 8, 12, 14, 16, 17, 18, X	+
SIR74ii (27)	1, 2, 4, 12, 14, 17, 18, 20, 21, 22, X	+
3W4 C15 (28)	7, 10, 11, 12, 14, 15, 17, 21, X	+
HORP27R C14 (29)	4, 7, 11, 12, 14, 15, 21, X	+
CTP41-2 (30)	2, 7, 8, 14, 16, 17, X	+
F4SC13 C19 (31)	1, 14, X	+
FIR5R3 (32)	14, 18	+
DT1.2R (25)	3, 10, 11, 17, 18, 20, 21, 22	−
HORL411B6P (29)	1, 3, 11, 18, 22, X	−
CI 21 (33)	7	−
Controls		
1R (28)	Mouse L-cell	−
Bu†	Human fibroblast	+

Note that several of the hybrids have been recloned and recharacterized since their description in the original reference. Human chromosomal constitution was determined by a combination of karyotypic[25], isozyme[36] and antigenic analysis[30]. Batches of cells for DNA production were checked by isozyme analysis.

*The hybridization conditions are given in Fig. 1 legend. Under conditions of high stringency only the 4.2- and 2.9-kb human bands were detected. In all cases where the hybrids were scored positive both bands were visible.

†A human fibroblast line derived from a human ovarian teratoma. This line was obtained from Dr S. Povey (MRC Biochemical Genetics Unit, London).

(Fig. 1*a*). This result is consistent with the nucleotide sequence of the human α-chain (ref. 10 and G.T. and S.T., unpublished observation), in which a *Hin*dIII site is located in the constant region, and suggests that a single constant-region gene segment is present in the human genome. One hybridizing band of 6 kb was detected with *Hin*dIII-digested mouse DNA probed at low stringency (data not shown). As the 4.2- and 2.9-kb bands were apparently specific to human DNA and the mouse-specific bands could be removed by washing at high stringency (Fig. 1*b*), the presence of the 4.2-kb and 2.9-kb fragments in *Hin*dIII-digested mouse/human somatic cell hybrid DNA could be used to determine the chromosomal location of the α-chain gene. Figure 1*c–f* shows Southern blotting analysis of DNA from four such hybrids. For hybrids positive for the human α-chain gene, both human-specific bands were always detected (see, for example, Fig. 1*c*, *e*). Inspection of the human chromosomal content of a panel of hybrids (Table 1) reveals that the human α-chain gene maps to chromosome 14. We, and others, have shown previously that the human β-chain gene family maps to chromosome 7 (refs 13, 14). Thus, the α- and β-chain genes lie on different chromosomes. The human β-chain genes also lie on a different chromosome from the immunoglobulin *Igh*, *Igk* and *Igl* loci. In contrast, the human α-chain gene is syntenic with the *Igh* loci, which have been localized in the region 14q32.33-14qter[16]. The human oncogene c-*fos* has also been localized to chromosome 14 in the region 14q21-q31[17].

A conserved linkage group between human chromosome 14 and mouse chromosome 14 occurs at the nucleoside phosphorylase (*Np*) locus (at 14q13.1 in human; reviewed in ref. 18). In man, nucleoside phosphorylase deficiency has been associated with severe T-cell immunodeficiency[19]. Interestingly, the gene encoding the mouse α-chain has been mapped to chromosome 14, close to the *Np-2* locus[20,21]. This observation suggests that the human α-chain gene may also lie close to the *Np* locus in a conserved linkage group. If this is the case, the human α-chain gene, although on the same chromosome, must reside a considerable distance from the *Igh* locus.

Abnormalities of chromosome 14 have been associated with human B-lymphocyte malignancies. Thus, Burkitt lymphomas are often associated with 8;14 translocations resulting in a juxtaposition of the c-*myc* and *Igh* loci[11]. Inversions of chromosome 14 due to breaks near q11 and q32 are found in T-cell chronic lymphocytic leukaemia and lymphomas, and simple translocations involving chromosome 14 have also been observed in T-cell malignancies[22–24]. For example, a translocation involving chromosomes 10 and 14 with a breakpoint at 14q11.2 has been found in a T-cell lymphoma cell line[24]. The involvement of the 14q11 region in these inversions and translocations suggests that it is related to T-cell function and that rearrangements involving this region may be involved in T-cell malignancies. If the human α-chain gene is located, by analogy with the linkage in the mouse, at or near the *Np* locus, it would be a candidate for such a gene. We are presently performing *in situ* hybridization analysis to test this possibility.

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1. Allison, J. P., McIntyre, B. W. & Bloch, D. *J. Immun.* **129**, 2293–2300 (1982).
2. Haskins, K. *et al. J. exp. Med.* **157**, 1149–1169 (1983).
3. Meuer, S. C. *et al. J. exp. Med.* **157**, 705–719 (1983).
4. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153–158 (1984).
5. Yanagi, Y. *et al. Nature* **308**, 145–149 (1984).
6. Chien, Y., Gascoigne, N. R. J., Kavaler, J., Lee, N. E. & Davis, M. M. *Nature* **309**, 322–326 (1984).
7. Siu, G. *et al. Cell* **37**, 393–401 (1984).
8. Saito, H. *et al. Nature* **312**, 36–40 (1984).
9. Chien, Y. *et al. Nature* **312**, 31–35 (1984).
10. Sim, G. K. *et al. Nature* **312**, 771–775 (1984).
11. Perry, R. P. *Cell* **33**, 647–650 (1983).
12. Lee, N. E. *et al. J. exp. Med.* **160**, 905–913 (1984).
13. Caccia, N. *et al. Cell* **37**, 1091–1099 (1984).
14. Collins, M. K. L. *et al. EMBO J.* **3**, 2347–2349 (1984).
15. Collins, M. K. L. *et al. EMBO J.* (submitted).
16. Croce, C. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 3416–3419 (1979).
17. Barker, P. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 5826–5830 (1984).
18. Ferguson-Smith, M. A. & Cox, D. R. *Cytogenet. Cell Genet.* **37**, 127–154 (1984).
19. Giblett, E. L., Ammann, A. J., Wara, D. W., Sandman, R. & Diamond, L. K. *Lancet* **i**, 1010–1013 (1975).
20. Dembić, Z., Bannwarth, W., Taylor, B. A. & Steinmetz, M. *Nature* **314**, 271–273 (1985).
21. Kranz, D. *et al. Science* (in the press).
22. Zech, L. *et al. Nature* **308**, 858–860 (1984).
23. Ueshima, Y., Rowley, J. D., Varikujis, D., Winter, J. & Gordon, L. *Blood* **63**, 1028–1038 (1984).
24. Hecht, E., Morgan, R., Hecht, B. K-M. & Smith, S. D. *Science* **226**, 1445–1447 (1984).
25. Solomon, E. *et al. Somat. Cell Genet.* **2**, 125–140 (1976).
26. Jones, E. A., Goodfellow, P. N., Kennett, R. H. & Bodmer, W. F. *Somat. Cell Genet.* **2**, 483–496 (1976).
27. Whitehead, A. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 5021–5025 (1982).
28. Nabholz, M., Giggiano, J. & Bodmer, W. F. *Nature* **223**, 358–363 (1969).
29. Van Heyningen, V. *et al. Ann. hum. Genet.* **38**, 295–303 (1975).
30. Tunnacliffe, A. & Goodfellow, P. in *Genetic Analysis of the Cell Surface, Receptors and Recognition* Vol. 16 (ed. Goodfellow, P.) 57–82 (Chapman & Hall, London, 1984).
31. Sykes, B. & Solomon, E. *Nature* **272**, 548–549 (1978).
32. Solomon, E. *et al. Cytogenet. Cell Genet.* **35**, 64–66 (1983).
33. Croce, C. M. & Koprowski, H. *J. exp. Med.* **140**, 1221–1229 (1974).
34. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
35. Feinberg, A. O. & Vogelstein, B. *Analyt. Biochem.* **137**, 266–267 (1984).
36. Harris, H. & Hopkinson, D. A. *Handbook of Enzyme Electrophoresis in Human Genetics* Suppl. 1977–78 (North-Holland, Amsterdam, 1976).

Stimulation of the Na/H exchanger of sea urchin eggs by phorbol ester

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On fertilization of a sea urchin egg, marked changes occur in the cytoplasmic concentration of calcium and hydrogen ions. These ionic signals represent the necessary and sufficient stimuli for the increased metabolism, protein synthesis and DNA synthesis that constitute egg activation[1–3]. Cytoplasmic alkalinization, the major immediate cause of the increased rate of protein synthesis which

occurs at fertilization[4], arises because the sperm-induced intracellular calcium transient activates a coupled flux of sodium ions and hydrogen ions across the oolemma[5–7]. The experiments reported here suggest that the second messenger which links the activation of the Na/H exchange to the calcium transient may be a substance which stimulates protein kinase C[8], as 12-*O*-tetradecanoyl phorbol acetate (TPA), a known activator of protein kinase C[9], appears to stimulate protein synthesis by turning on the Na/H exchanger and causing a cytoplasmic alkalinization. Our data indicate that one consequence of treating other tissues with TPA, a tumour promoter, may be an increase in intracellular *p*H[10–12].

The synthesis of proteins from maternal mRNA is necessary for the normal development of the sea urchin embryo[13]. Figure 1 shows that a concentration of TPA that supports proliferation in cultured mammalian cells[14] increased the rate of incorporation of amino acid into protein in unfertilized sea urchin eggs (*Lytechinus pictus*). The increased incorporation of amino acid into protein was slower in onset and of lesser magnitude than that stimulated at fertilization or by treatment with the calcium ionophore A23187 (refs 1, 4). Fertilization or treatment with ionophore stimulated a 10-fold increase in the rate of protein synthesis which is first detected 15 min after fertilization, whereas 320 nM TPA caused a fourfold increase in the rate after a lag of 30 min. At fertilization and on treatment with ionophore, calcium is released from a store within the egg and the cytoplasmic calcium concentration increases[15,16]. In contrast, treatment of eggs with up to 3 μM TPA caused none of the morphological changes associated with the increased cytoplasmic calcium concentration and consequent cortical granule exocytosis. The cortical granule exocytosis is a sensitive *in situ* assay for an increased intracellular calcium concentration[15,17]. As no cortical granule exocytosis occurred on treatment with TPA and since TPA itself does not, we have found, inhibit exocytosis *in vitro* or *in vivo* at these concentrations, we infer that no substantial increase in the calcium concentration, such as that which normally occurs at fertilization, occurred when the egg was treated with TPA.

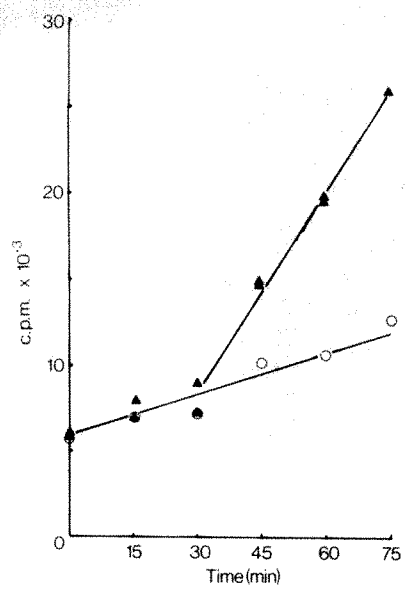


Fig. 1 TPA stimulates the incorporation of [4, 5-^{3}H]leucine into egg trichloroacetic acid (TCA) precipitates. Incorporation was measured using published methods[23]. Eggs were pre-loaded with radioactive amino acid and the TCA precipitate collected on glass-fibre filters. ▲, 320 nM TPA (Sigma) was added at $t = 0$ to eggs in artificial sea water (450 mM NaCl, 50 mM $MgCl_2$, 10 mM KCl, 11 mM $CaCl_2$, 2.5 mM $NaHCO_3$, *p*H 8.0). ○, Controls. The data shown are from two experiments. The mean increase in rate of [4, 5-^{3}H]leucine incorporation was 3.2 ± 0.1-fold (mean ± s.e.m., $n = 4$). TPA-treated eggs have an intact intracellular calcium store as they will raise normal fertilization membranes on insemination or addition of calcium ionophore. Temperature 16 °C.

Other parthenogenetic activators exist which stimulate protein synthesis in sea urchin eggs without releasing the egg's internal calcium store[4,18]. These are penetrating weak bases which stimulate protein synthesis by raising the egg's cytoplasmic *p*H[19]. Figure 2 presents data which indicate that TPA may be acting in a similar manner: unfertilized eggs loaded with fluorescein by incubation with fluorescein diacetate show a stable

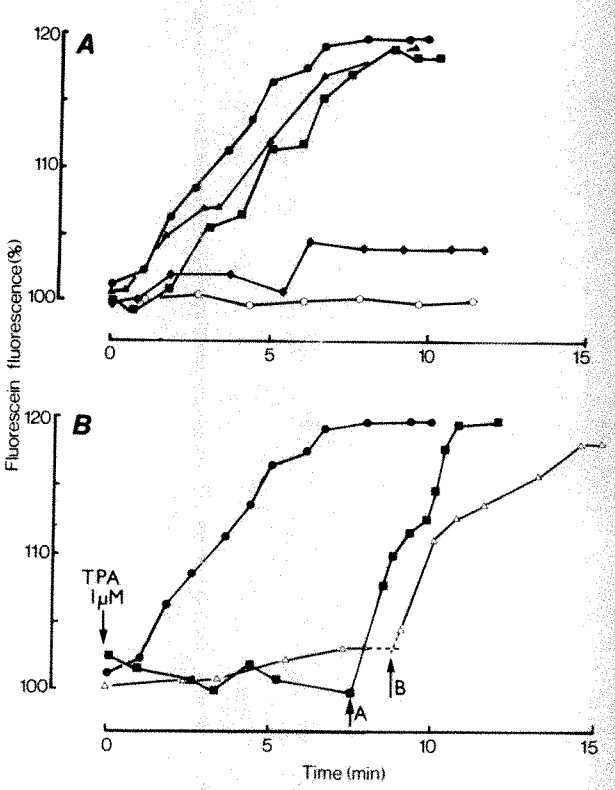


Fig. 2 The increase in fluorescein fluorescence in eggs treated with TPA and its less potent methyl analogue, 4-*O*-methyl-tetradecanoyl phorbol ester. Fluorescein fluorescence was measured in individual eggs using a Leitz Diavert fluorescence microscope and a photomultiplier as described previously[2]. *A*, Extent of the increase in fluorescein fluorescence at various concentrations of TPA: ●, 1,000; ▲, 200; ■, 100; ◆, 50 nM. The increase in fluorescence at maximal TPA concentrations was 117 ± 1.8% (mean ± s.e.m., $n = 9$). The increase in fluorescein fluorescence at fertilization in identical experimental conditions is 119 ± 3.1% (mean ± s.e.m., $n = 7$)[3]. To illustrate the pharmacological specificity of the response, open circles show the lack of response to the less potent analogue, MeTPA. *B*, Inhibition of the response to 1,000 nM TPA (●) by complete replacement of sodium in sea water by choline (■, $NaHCO_3$ was replaced with $KHCO_3$) or by addition of 100 μM dimethylamiloride (△). When sodium-containing sea water was re-admitted at the time indicated by the arrow labelled A, or dimethylamiloride removed at the time indicated by arrow B, the response was immediate.

Methods. Eggs were loaded with fluorescein by incubating them in sea water containing 50 μM fluorescein diacetate (Molecular Probes, Junction City, Oregon) for 3–5 min and washed three times before being lightly attached with polylysine to a coverslip on the microscope stage. Excitation was provided by a mercury vapour lamp provided with a 3 OD neutral density filter and 450–490-nm bandpass filter. Epifluorescent illumination was achieved with a 510-nm dichroic mirror. Emitted light was collected by a photomultiplier in the image plane provided with a 510-nm long-pass filter. The intensity of the 520-nm fluorescein fluorescence peak is sensitive to *p*H over the physiological range and provides information as to relative changes in *p*H. Exciting light was applied intermittently to minimize fluorescence bleaching. In these conditions the fluorescence signal remains constant for extended periods (> 1 h), indicating that the dye is neither lost from the eggs nor significantly bleached. We suppose that the fluorescence which we measure primarily reflects cytoplasmic *p*H, an assumption borne out by the similarities between the results obtained with this method and with that using intracellular *p*H microelectrodes[2]. Temperature 16 °C.

fluorescence signal[2] which increases when the eggs are treated with 100–1,000 nM TPA. The increase in fluorescein fluorescence is similar in magnitude to that seen at fertilization or after treatment with A23187, and can be attributed to an increase in cytoplasmic *p*H[2]. Though it might be argued that a proportion of the TPA-induced increase in fluorescein fluorescence is the result of fluorescein uptake into mitochondria[20], the absolute requirement for external sodium ions and the identical time course of the fluorescein increase and *p*H microelectrode potential under a variety of conditions[2] makes a substantial mitochondrial contribution to the fluorescence increase unlikely. That the TPA-induced increase in intracellular *p*H requires external sodium ions also suggests, by analogy with fertilization, that it is the exchange of external sodium ions for internal hydrogen ions that is responsible for the cytoplasmic alkalinization of TPA-treated eggs. This conclusion was confirmed by the finding that dimethylamiloride, a drug which specifically inhibits electroneutral Na/H exchange[21], inhibits the TPA-induced cytoplasmic alkalinization in eggs (see Fig. 3*B*).

The activation of the Na/H exchanger at fertilization leads to a transient net efflux of hydrogen ions from the egg[5–7]. Figure 3 shows that TPA induced an efflux of hydrogen ions that behaved very similarly to the hydrogen ion efflux which occurs at fertilization. Figure 3 also shows that 4-*O*-methyltetradecanoyl phorbol ester (MeTPA), a less potent TPA analogue, was ineffective at these concentrations. The TPA-induced efflux rate was maximal at 2.5 min and had fallen to control levels by 5 min after treatment. The efflux was sodium-dependent; it was sensitive to external sodium ion concentration in the range 0.1–5 mM at *p*H 8.0. This dependence on external sodium concentration is identical to that of the fertilization-induced Na/H exchange, which also requires only a small fraction of the normal sodium concentration of sea water for full activation[22]. The TPA-induced hydrogen ion efflux was half-maximal at 100 nM TPA, representing a net efflux of 20 nmol ml^{-1} of a 1% egg suspension at *p*H 8.0. The fertilization-induced efflux under similar conditions was 30 nmol ml^{-1} of a 1% egg suspension. The Na/H exchanger has been reported to contribute three-quarters of the total acid efflux occurring at fertilization[5] and is inhibited by amiloride[5,7]. We find that the TPA-induced hydrogen ion efflux is half-maximally inhibited by 40 μM dimethylamiloride, the concentration which half-maximally inhibits A23187-induced hydrogen ion efflux (see Fig. 3*B*). We conclude that TPA stimulates the Na/H exchanger of unfertilized eggs to extrude a quantity of hydrogen ions equivalent to the quantity extruded at fertilization. Activation of the Na/H exchange is a sufficient explanation of the effects of TPA on the intracellular *p*H.

A simple hypothesis for the mechanism by which TPA stimulates protein synthesis is that it acts primarily on the Na/H exchanger, and that it is the ensuing increase in intracellular *p*H which is responsible for the increase in the rate of incorporation of amino acid into protein. This simple hypothesis is supported by the data of Fig. 4. The increase in the rate of incorporation of amino acid into protein induced by TPA is similar to that induced by treatment with the weak base

Table 1 Effect of dimethylamiloride and removal of Na ions on TPA-induced increase in protein synthesis

	c.p.m. in protein at:	
	45 min	60 min
Control	2,110 ± 531	3,690 ± 284
320 nM TPA	4,783 ± 515*	5,265 ± 461†
320 nM TPA + 100 μM dimethylamiloride	2,773 ± 461 ($n = 3$)*	—
320 nM TPA in Na-free sea water	—	3,592 ± 702†

The c.p.m. values are the mean ± s.e.m. of four experiments of the type described in Fig. 1 legend.
* $P < 0.05$; † $P < 0.01$, Student's *t*-test.

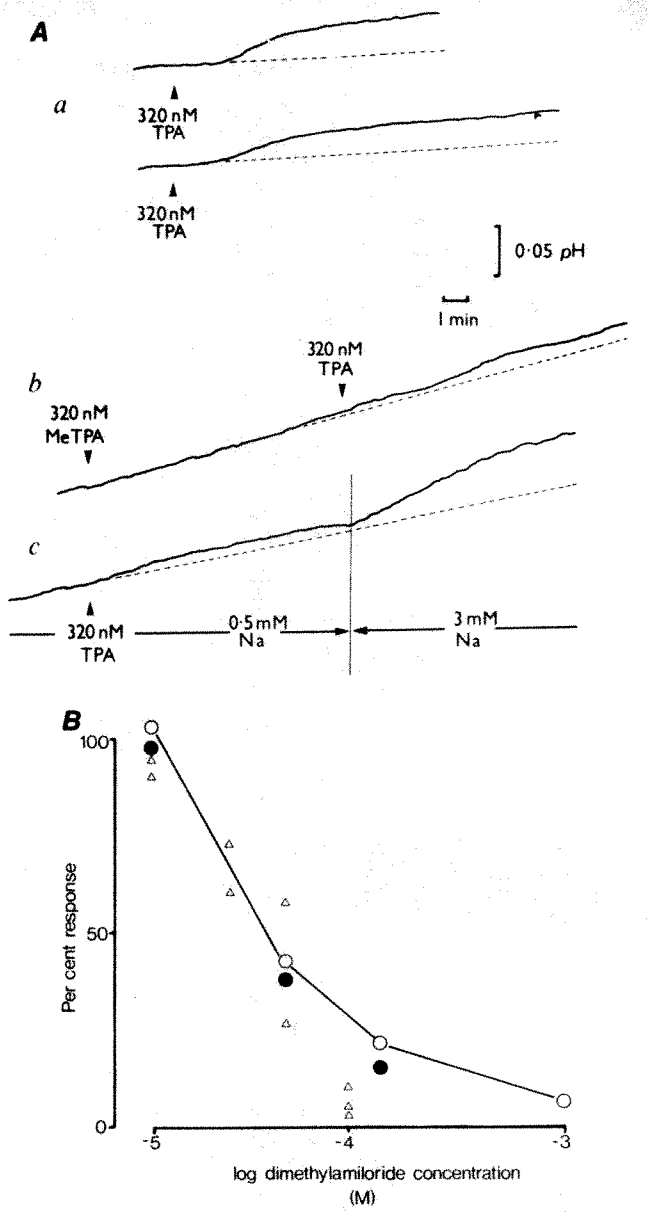


Fig. 3 The hydrogen ion efflux from unfertilized eggs measured with an external *p*H electrode in a 2% egg suspension in artificial sea water. The sea water was buffered normally and the external *p*H change kept to a minimum as the extent of the hydrogen ion efflux depends on the external *p*H[6]. ***A***: *a*, Two representative examples of the external acidification observed when the egg suspension was treated with 320 nM TPA (added from a 320 μM stock solution in dimethyl sulphoxide (DMSO); DMSO did not itself induce any of the changes shown in this or the other figures). The extent of the acid efflux was determined by back-titration with sodium hydroxide[5]. *b*, MeTPA (1,000 nM) caused no significant increase in acid efflux. A significant though attenuated response to 320 nM TPA was seen subsequently. *c*, The response to 320 nM TPA in artificial sea water containing 0.5 mM Na (choline-substituted) is one-fifth of the response in normal sea water. Further reduction in the sodium concentration completely abolishes the response (not shown). When sodium was added to increase the sodium concentration to 3 mM externally, the full TPA-induced hydrogen ion efflux was observed. The broken line is an extrapolation of the increase in extracellular *p*H which occurs in stirred, dense unfertilized egg suspensions in these conditions. Suspensions rapidly attained this linear rate of acid production, which was recorded for 5 min in each experiment before addition of the drug. ***B***, The TPA-induced hydrogen ion efflux is inhibited by dimethylamiloride[21] (●), as is the hydrogen ion efflux caused by the calcium ionophore A23187 (○). Also shown (△) is the inhibition by dimethylamiloride of the TPA-induced fluorescein fluorescence, measured as described in Fig. 2 legend. Sea water containing 50 mM sodium. Dimethylamiloride was given by Dr E. J. Cragoe Jr. Temperature 18 °C.

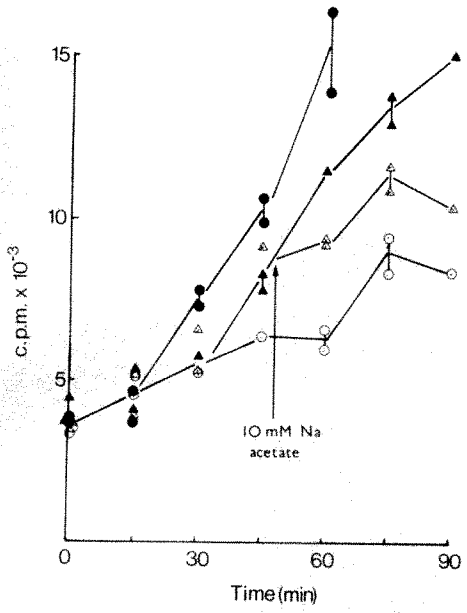


Fig. 4 TPA-induced protein synthesis and intracellular *p*H. The data are from two experiments. ●, 10 mM NH_4Cl induces a similar increase in the rate of protein synthesis to that obtained on treatment with 320 nm TPA (▲). When 10 mM sodium acetate in artificial sea water (*p*H 6.5)[19] is added to eggs which have been treated with 320 nM TPA (△), the rate of protein synthesis falls to that of controls (○). Conditions and methods are identical to those of the experiments of Fig. 1.

ammonium chloride, which is known to increase the rate of protein synthesis largely via its effects on intracellular *p*H[19]. Moreover, if the egg cytoplasm is made less alkaline after treatment with TPA by adding acetate, a weak acid[19], to the sea water in which the eggs are suspended, then the rate of protein synthesis falls back to the rate seen in the untreated egg. Finally, Table 1 shows that the TPA-induced increase in protein synthesis is prevented by the presence of 100 μM dimethylamiloride or by the removal of sodium ions from the sea water in which the eggs are suspended. These data indicate that the TPA-induced increase in intracellular *p*H is a necessary stimulus for protein synthesis.

Our results strongly suggest that TPA stimulates protein synthesis in unfertilized sea urchin eggs by activating a Na/H exchange at the oolemma, which in turn leads to a cytoplasmic alkalinization similar in magnitude to that observed at fertilization. We believe that these effects occur without the participation of a calcium transient of the magnitude observed at fertilization, though we cannot exclude the possibility that small alterations in cytoplasmic calcium occur. The absence of a calcium transient in TPA-treated eggs accounts for the disparity between the rates of protein synthesis in fertilized and TPA-treated eggs: it has been shown that maximal rates of protein synthesis are achieved only if both the calcium concentration and intracellular *p*H increase[23]. The prevailing idea is that many of the actions of TPA may be explained by its ability to promote phosphorylation of specific membrane proteins through activation of a membrane protein kinase, protein kinase C[9,24]. Protein kinase C is thought to be regulated *in vivo* by diacylglycerol, one of the two putative second messengers generated by the hydrolysis of plasma membrane polyphosphoinositides[25]. We have shown that the other second messenger, inositol trisphosphate, will activate eggs and that physiological calcium concentrations promote the hydrolysis of egg membrane polyphosphoinositides[26]. We suggest that diacylglycerol may be the second messenger that links the stimulation of the Na/H exchange to the calcium transient of the egg at fertilization and so promotes mitosis and cell division.

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Note added in proof: A stimulation of Na/H exchange by phorbol ester and diacylcylcerol has recently been reported to occur in cells in culture[27].

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1. Steinhardt, R. A. & Epel, D. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1915–1919 (1974).
2. Whitaker, M. J. & Steinhardt, R. A. *Cell* **25**, 95–103 (1981).
3. Whitaker, M. J. & Steinhardt, R. A. *Q. Rev. Biophys.* **15**, 593–666 (1982).
4. Epel, D., Steinhardt, R. A., Humphreys, T. & Mazia, D. *Devl Biol.* **40**, 245–255 (1974).
5. Johnson, J. D., Epel, D. & Paul, M. *Nature* **262**, 661–664 (1976).
6. Cuthbert, A. & Cuthbert, A. W. *Expl Cell Res.* **114**, 409–415 (1978).
7. Payan, P., Girard, J.-P. & Ciapa, B. *Devl Biol.* **100**, 29–38 (1983).
8. Nishizuka, Y. *Phil. Trans. R. Soc.* **B302**, 101–112 (1983).
9. Castagna, M. *et al. J. biol. Chem.* **257**, 7847–7851 (1982).
10. Dicker, P. & Rosengurt, E. *Biochem. biophys. Res. Commun.* **100**, 433–441 (1981).
11. Besterman, J. M. & Cuatrecasas, P. *J. Cell Biol.* **99**, 340–343 (1984).
12. Whiteley, B., Cassel, D., Zhuang, Y.-X. & Glaser, L. *J. Cell Biol.* **99**, 1162–1166 (1984).
13. Timourian, H. & Uno, J. *Expl Cell Res.* **48**, 173–176 (1967).
14. Hecker, E. (ed.) *Cocarcinogenesis and Biological Effects of Tumour Promoters* (Raven, New York, 1982).
15. Steinhardt, R. A., Zucker, R. & Schatten, G. *Devl Biol.* **58**, 185–196 (1977).
16. Zucker, R. S., Steinhardt, R. A. & Winkler, M. M. *Devl Biol.* **68**, 285–295 (1978).
17. Baker, P. F. & Whitaker, M. J. *Nature* **276**, 513–515 (1978).
18. Vacquier, V. D. & Brandriff, B. *Devl Biol.* **47**, 12–31 (1975).
19. Winkler, M. M. & Grainger, J. L. *Nature* **273**, 236–248 (1978).
20. Thomas, J. A., Buchsbaum, R. N., Zimniak, A. & Racker, E. *Biochemistry* **18**, 2210–2218 (1979).
21. Vigne, P., Frelin, C., Cragoe, E. J. Jr & Lazdunski, M. *Molec. Pharmac.* **25**, 131–136 (1984).
22. Shen, S. S. & Steinhardt, R. A. *Nature* **282**, 87–89 (1979).
23. Winkler, M. M., Steinhardt, R. A., Grainger, J. L. & Minning, L. *Nature* **287**, 558–560 (1980).
24. Nishizuka, Y. *Nature* **308**, 593–698 (1983).
25. Berridge, M. J. *Biochem. J.* **220**, 345–360 (1984).
26. Whitaker, M. J. & Irvine, R. F. *Nature* **312**, 636–639 (1984).
27. Moolenaar, W. H., Tertoolen, L. G. J. & de Laat, S. W. *Nature* **312**, 371–374 (1984).

Focus formation in rat fibroblasts exposed to a tumour promoter after transfer of polyoma *plt* and *myc* oncogenes

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The gene encoding the large-T protein of polyoma virus (*plt*), the E1A genes of adenoviruses, the viral *myc* gene (v-*myc*) or rearranged forms of the cellular c-*myc* gene confer on rat embryo fibroblast (REF) cells in primary culture a series of new properties ('immortality', reduced serum requirement and sensitivity to transformation by viral and activated cellular oncogenes) but do not induce the appearance of transformed foci[1–6]. We now report that focus formation can be induced after transfer of these genes into either REF or established FR3T3 rat cells by subsequent exposure to the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA)[7,8]. Frequencies of transformation are in the same range as those usually observed for transformation with complete polyoma DNA or with a mixture of cloned *myc* and *ras* oncogenes. These results further characterize the 'immortalized' state induced by *plt* and *myc* as one in which the cells maintain a normal growth control in many respects but can be further acted upon to produce a neoplastic progeny.

Complete transformation of REF cells first requires the establishment of immortalization, a complex phenotype induced by *plt*, adenovirus E1A or v-*myc* and c-*myc* oncogenes (group I). The activity of these genes allows subsequent transformation by a second class of oncogenes (group II) which includes polyoma virus *pmt* (middle-T protein), adenovirus E1B, retroviral and activated cellular *ras* genes[2–5]. Transfer of *plt*, rearranged *myc* or adenovirus E1A genes into REF cells results in all cases in the same alterations of growth control in culture,

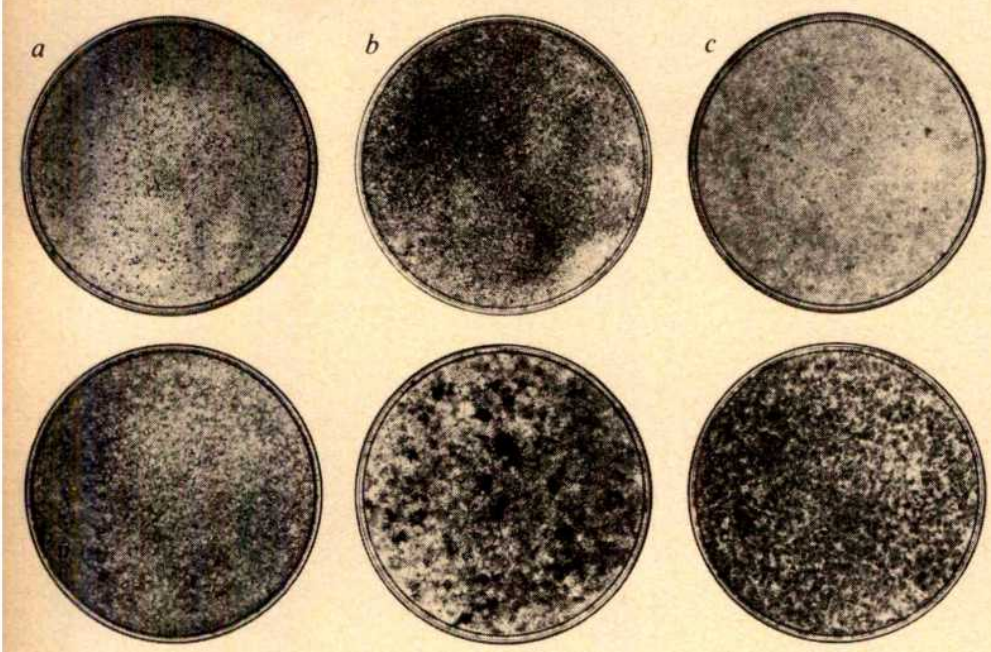


Fig. 1 Effect of TPA on FR3T3 cells immediately after transfer of the *plt* gene and on FR3T3-LT1 cells, which express constitutively the polyoma virus large-T protein[1]. Experimental conditions are described in the text and in Table 1 legend. *a*, FR3T3; *b*, FR3T3 after transfer of plasmid pPyLT1; *c*, FR3T3-LT1. Upper row, control cultures treated with DMSO alone; bottom row, TPA-treated cultures.

most characteristically the acquisition of unlimited growth ability and reduced requirements for serum factors (refs 2, 6 and our unpublished results). Apart from these changes, immortalized cells retain the low saturation density and anchorage dependence of normal fibroblasts and thus cannot be selected by the usual transformation procedures (focus formation, growth in suspension). However, although still normal in many respects, *plt*- and *myc*-immortalized lines exhibit an increased probability of further oncogenic transformation. Tumour promoters transform cells which have first undergone an 'initiation' event[7,8] induced by carcinogens, which have been shown independently to immortalize rodent embryo fibroblasts[9,10]. We therefore investigated whether cells immortalized by *plt* or *myc* could be transformed further by treatment with TPA.

The effect of a 12-day exposure to TPA (2×10^{-5} M in 0.01% dimethyl sulphoxide, DMSO) after transfer of the polyoma *plt* gene was first studied in REF cells. Control cultures either were not treated at all or were treated after gene transfer with DMSO in the absence of TPA or were treated only with TPA. Foci developed in cultures treated with TPA after gene transfer (data not shown), but precise quantitation was hampered by the background of cells growing past confluency in primary REF cells, especially after treatment with TPA, which is known to have mitogenic effects[11]. Selection for colonies in agarose-gelified medium, however, produced unambiguous results (Table 1). Whereas neither treatment with TPA nor transfer of *plt* alone led to colony formation, the combination of the two treatments resulted in the appearance of clones that produced large colonies in suspension after the removal of TPA. Establishment of cell lines from such colonies (not shown) confirmed that they stably maintained their transformed phenotype. Transfer of the polyoma *pmt* gene (middle-T protein), with or without TPA treatment, conferred on REF cells only the ability to grow for a limited number of generations in suspension, as expected from the absence of an immortalization function.

We then investigated whether TPA produced the same effect in cells of the established line FR3T3. This would imply that the spontaneous immortalization event that gave rise to the line is not equivalent to immortalization by *plt* or *myc* genes, as has been suggested for FR3T3 rat cells[6] and BALB/c3T3 mouse cells[12] by studies on the reduced requirement for serum growth factors in culture which is conferred by group I oncogenes. TPA treatment, in the same conditions as in the previous experiments, did not affect FR3T3 cell growth; however, following transfer of the *plt* gene, foci appeared within 4–6 weeks of TPA removal (Fig. 1). The same result was obtained when *plt* was replaced by the viral *myc* gene or by a rearranged mouse c-*myc* gene (carried on plasmids pSV-v-myc and pSV-c-myc-1, respectively[4]; Table 2). The numbers of foci per plate after *plt* and TPA treatment were in the same range as obtained after transfer of

Table 1 Colony formation in agarose medium by REF cells after transfer of isolated polyoma genes and subsequent treatment with TPA

Transfer of plasmid	Viral protein	TPA treatment	No. of colonies per plate (2×10^5 cells)	Size of colonies
None	–	–	0, 0, 0	–
None	–	+	0, 0, 0	–
pPyLT1	Large-T	–	0, 0, 0	–
pPyLT1	Large-T	+	2, 5, 8, 1, 3, 10	$>10^4$ cells*
pPyMT1	Middle-T	+	21, 13, 10, 17, 25, 19	<50 cells

REF secondary cultures were prepared as described previously[2] and DNA of plasmids pPyLT1 (*plt* gene) and pPyMT1 (*pmt* gene)[14,15] transferred by the protoplast fusion technique also as described elsewhere[1]. Cells were maintained for a total of 12 days with three medium changes in DMEM supplemented with 10% newborn calf serum (Gibco) and containing either 2×10^{-5} M TPA (Sigma) in 0.01% DMSO or 0.01% DMSO without TPA. Cells were then trypsinized and seeded in agarose medium[1] at a density of 2×10^5 cells per plate (three to six identical plates). The number of colonies in each plate was counted 3 weeks later.

* Macroscopically visible colonies.

Table 2 Focus formation by FR3T3 cells after transfer of *plt* and *myc* genes and subsequent treatment with TPA

Transfer plasmid	TPA treatment	Foci per plate (2×10^5 cells)
None	–	0, 0, 0
None	+	0, 0, 0
pPyLT1	–	0, 0, 2
pPyLT1	+	7, 15, 18
pSV-v-myc	–	0, 0, 1
pSV-v-myc	+	3, 4, 9
pSV-c-myc-1	–	0, 0, 0
pSV-c-myc-1	+	16, 22, 24

DNA of plasmids pPyLT1[15], pSV-v-myc and pSV-c-myc-1[4] was transferred into FR3T3 cells by the protoplast fusion technique as described in ref. 1 and TPA treatment was applied as described in Table 1 legend. Foci were counted following Giemsa staining after 6 weeks.

Table 3 Stability of the transformed phenotype during prolonged growth in the absence of TPA in cell lines derived from foci induced by TPA after transfer of the *plt* and *myc* oncogenes

Cell line	Plating efficiency in agarose medium (%)
FR-PLTTPA-32	10
FR-CMYCTPA-3	14
FR-VMYCTPA-3	8
FR3T3	<0.001

Cell lines FR-PLTTPA-32, FR-VMYCTPA-3 and FR-CMYCTPA-3 were isolated after transfer of plasmids pPyLT1, pSV-v-myc and pSV-c-myc-1, respectively, and subsequent TPA treatment as described in Table 2. For focus formation, the cultures had been maintained in DMEM without TPA for 4 weeks. Cells picked from foci were then grown in the same medium for three successive passages (~10 generations), still in the absence of TPA, and eventually seeded in agarose-gelified DMEM (2×10^5 cells per plate), in parallel with control FR3T3 cells. The number of colonies per microscope field, counted after 1 week, is expressed as a percentage of the input.

a wild-type polyoma genome expressing both the *plt* and *pmt* genes (data not shown; see ref. 1). When cells picked from such foci were grown further in Dulbecco's modified Eagle's medium (DMEM) in the absence of TPA, they still exhibited the characteristic morphology of transformed cells, and their efficiency of plating in agarose medium (10–20%) was comparable with that of typical virus-transformed lines[13] (Table 3). Cells that permanently expressed immortalizing oncogenes were also treated with TPA. Such cell lines had been derived previously from FR3T3 cells after transfer of *plt* and *myc* and selection by colony formation in medium supplemented only with 0.5% serum[1,6]. Unlike the original FR3T3 cells, such cultures reacted to TPA treatment by a dramatic change in their growth properties, showing an increase in saturation density within 2 weeks of TPA removal (Fig. 1).

Expression of polyoma virus *plt*, v-*myc* and rearranged c-*myc* genes therefore not only confers on normal rodent fibroblasts a set of new growth properties, such as immortalization or serum independence, but also allows further transformation by activated *ras* and polyoma *pmt* genes[1–6]; these genes bring the cells into a state where they now respond to environmental stimuli that do not induce permanent transformation of the original normal cell. Our results further demonstrate that the phenotype induced by group I oncogenes is not equivalent to that of the spontaneously immortalized 3T3 cells, as previously indicated by studies on growth factor requirements[6,12]. From a practical viewpoint, we note that, as found previously using other biological assays[6,12], gene transfection combined with TPA treatment may provide a means of discovering new oncogenes of this group possibly activated in human and experimental tumours.

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1. Rassoulzadegan, M. *et al. Nature* **300**, 713–718 (1982).
2. Rassoulzadegan, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4354–4358 (1983).
3. Van den Elsen, P. J., de Pater, S., Houweling, A., van der Veer, J. & van der Eb, A. *Gene* **18**, 175–185 (1982).
4. Land, H., Parada, L. F. & Weinberg, R. A. *Nature* **304**, 596–602 (1983).
5. Ruley, H. E. *Nature* **304**, 602–606 (1983).
6. Mougneau, E., Lemieux, M., Rassoulzadegan, M. & Cuzin, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5758–5762 (1984).
7. Beremblum, I. *Cancer Res.* **1**, 44–48 (1941).
8. Heidelberger, C. *A. Rev. Biochem.* **44**, 79–111 (1975).
9. Newbold, R. F. & Overell, R. W. *Nature* **304**, 648–651 (1983).
10. Newbold, R. F., Overell, R. W. & Connell, J. R. *Nature* **299**, 633–635 (1982).
11. Weinstein, B., Lee, L. S., Fisher, P. B., Mufson, A. & Yamasaki, H. *J. supramolec. Struct.* **12**, 195–208 (1979).
12. Armelin, H. A. *et al. Nature* **311**, 655–660 (1984).
13. Rassoulzadegan, M., Perbal, B. & Cuzin, F. *J. Virol.* **28**, 1–5 (1978).
14. Tyndall, C., La Mantia, G., Thacker, C. M., Favaloro, J. & Kamen, R. *Nucleic Acids Res.* **9**, 6231–6250 (1981).
15. Treisman, R., Novak, U., Favaloro, J. & Kamen, R. *Nature* **292**, 595–600 (1981).

Independent effects of growth hormone releasing factor on growth hormone release and gene transcription

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The synthesis and secretion of growth hormone (GH) by the somatotropic cells of the anterior pituitary is under complex hormonal regulation. The hypothalamic peptides, growth hormone releasing factor (GHRF) and somatostatin, respectively stimulate[1–3] and block[4] GH release. We have shown that treatment of primary cultures of rat anterior pituitary cells with GHRF stimulates transcription of the GH gene, as well as GH release, and that this reflects the response of pituitary cells *in vivo*[5]. Gick *et al.* have shown a stimulation of GH mRNA levels in normal pituitary cells in response to GHRF[6]. Because normal pituitary cells are the physiological target for GHRF and because they exhibit both transcriptional and release responses to the hormone, they provide a valuable system with which to examine the important possibility of a link between hormone synthesis and release in secretory cells. Using this system, we demonstrate here that GHRF stimulates GH gene transcription independently of GH release and, conversely, that other agents can stimulate GH release without affecting transcription of the GH gene.

Treatment of primary cultures of pituitary cells with GHRF leads to a rapid rise in intracellular levels of cyclic AMP[7]. This event, which has been previously shown to stimulate the release of GH from pituitary cells[8–12], suggests that the effects of GHRF are mediated, at least in part, by a cyclic AMP-dependent mechanism. GHRF- or cyclic AMP-stimulated GH release is blocked by agents that block voltage-dependent calcium channels, such as verapamil[7–12], demonstrating that the release of stored GH is calcium-dependent. Therefore, it seems that the sequence of events leading to GHRF-induced GH release includes both a rise in cyclic AMP levels and a Ca^{2+}-dependent step that causes GH release. Our experiments were designed to investigate the possible link between GHRF-stimulated GH release and transcription, by determining which, if any, of the events leading to GH release are also involved in stimulation of GH gene transcription. We measured GH-specific transcription and GH release, as well as intracellular cyclic AMP levels, for each treatment described. Cell treatments were performed for 1 h, at which time nuclei were collected, and GH-specific transcription was determined by nuclear run-off assay[5]. GH release and intracellular levels of cyclic AMP were monitored by radioimmunoassay (RIA).

To determine whether release of stored hormone is required for the transcriptional stimulation by GHRF, we used several treaments to block GH release. We performed experiments in media containing either 2.5 mM EGTA, to chelate Ca^{2+}, or 100 μM verapamil, to block voltage-dependent Ca^{2+} channels. In both cases, GHRF induction of GH release was blocked, but stimulation of GH gene transcription and intracellular cyclic AMP levels were not diminished (Fig. 1). Treatment with 10 nm somatostatin lowered basal GH release as well as GHRF-stimulated GH release (Fig. 2). The rise in cyclic AMP was diminished by somatostatin, as expected[7] (60% reduction), but there was no inhibitory effect on basal, or GHRF-stimulated, GH gene transcription. These results indicate that the transcriptional response to GHRF is not dependent on GH release or on extracellular Ca^{2+}.

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Although the above experiments show that GH release is not required for GHRF stimulation of GH gene transcription, they do not address the possibility that GH release alone can stimulate transcription of the GH gene. To examine this possibility directly, we used several different treatments to stimulate GH secretion. Depolarizing concentrations of K^+ have been shown to release GH from pituitary cells[13,14]. A 1-h treatment with 58 mM K^+ caused GH release without affecting either intracellular cyclic AMP levels or the rate of transcription of the GH gene (Fig. 3). Treatment of pituitary cells with the tumour promoter, phorbol myristate acetate (PMA)[15,16], caused GH release[17], but failed to stimulate cyclic AMP production or GH gene transcription (Fig. 3). Forskolin, a diterpene compound

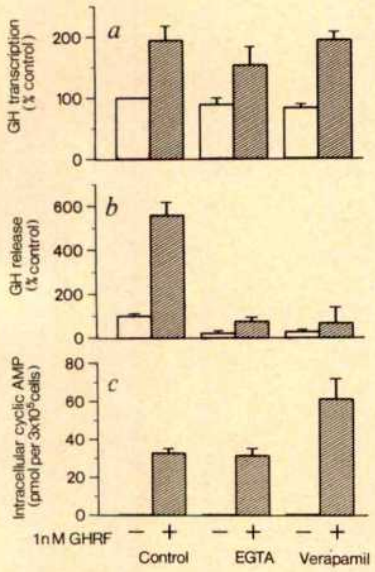


Fig. 1 Effects of EGTA and verapamil on GHRF stimulation of GH gene transcription (*a*), GH release (*b*), and intracellular cyclic AMP levels (*c*). Parallel plates of cells were treated with 1 nM GHRF (+) or GHRF dilution buffer (−). Where indicated, the medium contained 2.5 mM EGTA or 100 μM verapamil. GH-specific transcription and GH release were determined from the same experiment and are expressed as % of control. Cyclic AMP was measured in a separate parallel experiment and is expressed as pmol cyclic AMP per 3×10^5 cells.

Methods. *a*, *b*, Primary cultures of anterior pituitary cells were prepared as described previously[5,25,26] and plated at a density of 5×10^6 cells per 60-mm dish in medium containing 5 nM dexamethasone and 30 pM triiodothyronine. After 3 days in culture, the cells were washed by multiple medium change with B-PJ medium containing 0.1% bovine serum albumin and 2.5×10^{-4} M ascorbic acid[26] and returned to the incubator. After 1 h of incubation, they were treated with 1 nM rat hypothalamic GHRF (rhGHRF, synthesized by J. Rivier[3]) or a comparable volume of GHRF dilution buffer (1 mM acetic acid), in fresh B-PJ medium (control) or B-PJ containing 2.5 mM EGTA or 100 μM verapamil. After 1 h of treatment, the medium was removed from the cells and assayed for immunoreactive GH by RIA[7]. Cells were lysed, nuclei were prepared and elongation of nascent RNA chains was carried out as previously described[5,27]. RNA was prepared [27] from the nuclei and hybridized to excess plasmid DNA immobilized on nitrocellulose filters. The filters were washed, RNase-treated, dried and counted in toluene scintillation fluid. GH gene transcription was measured by quantifying RNase-resistant hybridization to a full-length GH cDNA clone. Background hybridization was determined by hybridization to pBR322 and the background values were subtracted from the GH hybridization values to give specific hybridization. GH-specific hybridization was determined as p.p.m. of total input RNA, and expressed as % of control (mock-treated). In reporting the data, no corrections were made for the size of the hybridization probe or the efficiency of hybridization. Each point represents treatment of a single plate of cultured cells and all experiments were repeated at least twice. Hybridizations were done in triplicate and data are presented as the mean ±s.e. for triplicate hybridizations from two similar experiments. *c*, Cells were prepared as in *a*, except that they were plated at 3×10^5 cells per well, in Linbro 24-well Petri dishes. Cell treatments were carried out as in *a*. After 1 h of treatment, cells were processed for cyclic AMP extraction as described previously[28]. All treatments were carried out in triplicate wells. Cyclic AMP was assayed[7] by RIA.

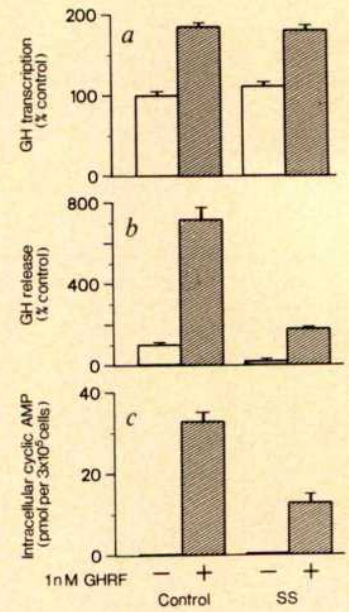


Fig. 2 Effect of somatostatin (SS) on GHRF stimulation of GH gene transcription (*a*), GH release (*b*), and intracellular cyclic AMP levels (*c*). Parallel plates of cells were treated with 1 nM GHRF (+) or dilution buffer (−), in the presence or absence of 10 nM SS in the medium.

Methods. Same as for Fig. 1. The experiment was repeated three times with similar results, and the data shown are from one representative experiment.

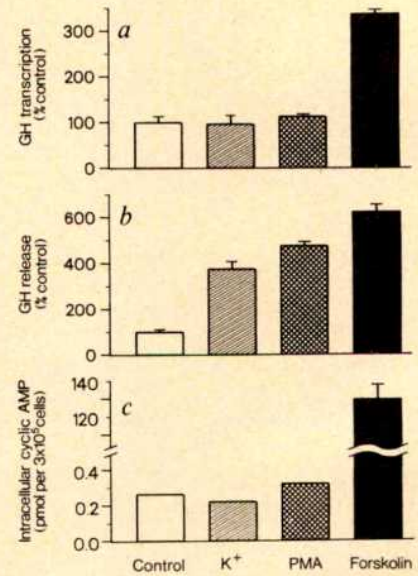


Fig. 3 Effects of K^+, PMA and forskolin on GH gene transcription *a*, GH release *b*, and intracellular cyclic AMP levels *c*. Parallel plates of cells were incubated in the presence or absence of 58 mM K^+, 100 nM PMA, or 10 μM forskolin. In *a* and *b*, values for each treatment are expressed as per cent of the control value for that treatment. In *c*, absolute values are shown. Controls for *c* did not differ significantly.

Methods. Same as for Fig. 1, except that K^+ treatment was carried out in Krebs' Ringer's buffer with 11 mM glucose. Treatments were performed at least twice with similar results, and results reported are from one representative experiment. Where error bars are not shown, the standard error was too small to plot.

that activates the catalytic subunit of adenylate cyclase[18], also stimulated GH release, but, unlike K^+ and PMA, forskolin caused a rise in intracellular cyclic AMP levels and stimulated GH gene transcription also (Fig. 3).

Exposure of pituitary cells to GHRF provokes several cellular responses, including a rise in intracellular cyclic AMP levels and a Ca^{2+}-dependent step that leads to GH release. We have shown that GH release is not the stimulus for the increase in transcription, by demonstrating that the transcriptional induction occurs in conditions that inhibit GH release, and that two treatments that stimulate GH release in the absence of GHRF have no effect on transcription of the GH gene. As GHRF stimulation of GH gene transcription is not dependent on external Ca^{2+}, it also follows that the Ca^{2+}-dependent step in the release response is not the stimulus for the transcriptional response.

As the physiological inhibitor of GHRF action, it might be expected that somatostatin would block the transcriptional effects of GHRF. Somatostatin reduces, but does not eliminate, the GHRF-stimulated rise in cyclic AMP[7], and has been shown to activate the N_i (inhibitory) subunit of adenylate cyclase[19]; but there is much evidence to suggest that somatostatin exerts another inhibitory effect on GH release at the calcium-dependent step(s) in the release pathway, distal to the elevation of cyclic AMP levels. Somatostatin blocks stimulation of GH release by cyclic AMP derivatives[20] and agents that raise cyclic AMP levels[7,11], as well as treatments which cause influx of Ca^{2+} (refs 13, 14). Furthermore, Stachura has proposed that somatostatin has no effect on GH synthesis, based on the observation that dibutyryl cyclic AMP causes a build-up of stored GH behind a somatostatin block[21,22]. Our results show that somatostatin inhibits GH release without influencing GHRF-stimulated transcription of the GH gene, which is consistent with Stachura's model.

Of the agents we used to stimulate GH release in the absence of GHRF, forskolin caused a rise in cylic AMP levels, whereas PMA and K^+ did not. Of the three agents, only forskolin stimulates GH gene transcription. These data, together with our observation that neither EGTA, verapamil, nor somatostatin eliminates the GHRF-induced rise in cyclic AMP, suggest a link between the rise in intracellular cyclic AMP levels and stimulation of GH gene transcription. If cyclic AMP were responsible for the stimulation of GH gene transcription, such an effect might be mediated by phosphorylation of putative nuclear regulatory proteins. Phosphorylation of nuclear proteins has been associated with the stimulation of prolactin transcription by thyrotropin-releasing hormone[23] and cyclic AMP[24]. In our system we have observed phosphorylation of H1 histone at serine 37 in response to treatment with GHRF or 8-bromo cyclic AMP (M.B. and M. Waterman, unpublished observations). Taken together, the data presented here clearly show that GHRF stimulation of GH gene transcription occurs independently of GH release and suggest a role for cyclic AMP as the mediator of the transcriptional stimulation.

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1. Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300**, 276–278 (1982).
2. Guillemin, R. *et al. Science* **218**, 585–587 (1982).
3. Spiess, J., Rivier, J. & Vale, W. *Nature* **303**, 532–535 (1983).
4. Brazeau, P. *et al. Science* **179**, 77–79 (1973).
5. Barinaga, M. *et al. Nature* **306**, 84–85 (1983).
6. Gick, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 1553–1555 (1984).
7. Bilezikjian, L. M. & Vale, W. W. *Endocrinology* **113**, 1726–1731 (1983).
8. Schofield, J. G. *Nature* **215**, 1382–1383 (1967).
9. Cooper, R. H., McPherson, M. & Schofield, J. G. *Biochem. J.* **127**, 143–154 (1972).
10. Sundberg, D. K., Fawcett, C. P. & McCann, S. M. *Proc. Soc. exp. Biol. Med.* **151**, 149–154 (1976).
11. Sheppard, M. S., Spence, J. W. & Kraicer, J. *Endocrinology* **105**, 261–268 (1979).
12. Spence, J. W., Sheppard, M. S. & Kraicer, J. *Endocrinology* **106**, 764–769 (1980).
13. Kraicer, J. & Spence, J. W. *Endocrinology* **108**, 651–657 (1981).
14. Kraicer, J. & Chow, A. E. H. *Endocrinology* **111**, 1173–1180 (1982).
15. Castagna, M. *et al. J. biol. Chem.* **257**, 7847–7851 (1982).
16. Niedel, J. E., Kuhn, L. J. & Vandenbark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 36–40 (1983).
17. Smith, M. & Vale, W. *Endocrinology* **107**, 1425–1431 (1980).
18. Seamon, K. B. & Daly, J. W. *J. Cyclic Nucleotide Res.* **7**, 201–224 (1981).
19. Jakobs, K. H., Aktories, K. & Schultz, G. *Nature* **303**, 177–178 (1983).
20. Vale, W. *et al. C. r. hebd. Séanc. Acad. Sci., Paris Ser. D* **275**, 2913–2916 (1972).
21. Stachura, M. *Endocrinology* **101**, 1044–1053 (1977).
22. Stachura, M. *Endocrinology* **108**, 1027–1034 (1981).
23. Murdoch, G. H., Franco, R., Evans, R. M. & Rosenfeld, M. G. *J. biol. Chem.* **258**, 15329–15335 (1983).
24. Murdoch, G. H., Rosenfeld, M. G. & Evans, R. M. *Science* **218**, 1315–1317 (1982).
25. Vale, W., Grant, G., Amoss, M., Blackwill, R. & Guillemin, R. *Endocrinology* **91**, 562–572 (1972).
26. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J. & Rivier, J. *Endocrinology* **112**, 1553–1555 (1983).
27. Evans, R. M., Birnberg, N. C. & Rosenfeld, M. G. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7659–7663 (1982).
28. Bilezikjian, L. M. & Vale, W. W. *Endocrinology* **113**, 657–662 (1983).

Growth induced by pulsatile infusion of an amidated fragment of human growth hormone releasing factor in normal and GHRF-deficient rats

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The discovery of human pancreatic growth hormone releasing factors (GHRFs)[1,2] and subsequent characterization of human hypothalamic GHRF[3] has led to studies on the role of these peptides in stimulating growth hormone (GH) release[4–6], and attempts to use GHRF peptides to increase growth rates in short children are already underway[7]. However, there is no experimental evidence in animals that exogenous GHRF promotes growth *in vivo*. Although anaesthetized rats release GH reproducibly in response to GHRF injections[4,8], the responses in conscious male rats are much more variable[5,6], perhaps because of their highly episodic endogenous GH secretory pattern[9]. In contrast, female rats secrete GH in a more continuous pattern[10] and respond reproducibly to repeated injections of GHRF. We report here that it is possible to establish a 'male' type of GH secretory pattern in normal female rats by long-term pulsatile intravenous (i.v.) infusions of the active human GHRF fragment GHRF (1–29)NH_2. We found that this treatment accelerates growth and increases pituitary GH content, whereas continuous infusions of this GHRF fragment at the same daily dose are ineffective. Pulsatile, but not continuous GHRF also stimulates growth in animals made GHRF-deficient by neonatal monosodium glutamate treatment. Thus exogenous GHRF will stimulate growth in both GHRF-deficient and normal animals provided it is administered in an appropriate pattern.

Injections of hGHRF(1–29)NH_2 (1 μg) were given i.v. every 3 h to a group of six conscious female rats (Fig. 1*a*). This treatment produced plasma GH profiles in female rats which were very similar to the endogenous GH secretory pattern in male rats (Fig. 1*b*). There was no evidence of endogenous secretory episodes between the exogenously induced GH pulses, and all the animals became entrained, responding uniformly to successive injections with peak plasma GH levels exceeding 1 μg ml^{-1}. Using a chronic i.v. infusion system with programmable multi-channel infusion pumps[11,12], groups of eight female rats were given saline or hGHRF(1–29)NH_2 either as a continuous infusion (8 μg per day) or as 1-μg pulses every 3 h for 12 days. The ability of this long-term pulsatile GHRF treatment to elicit pulses of GH secretion was assessed by withdrawing

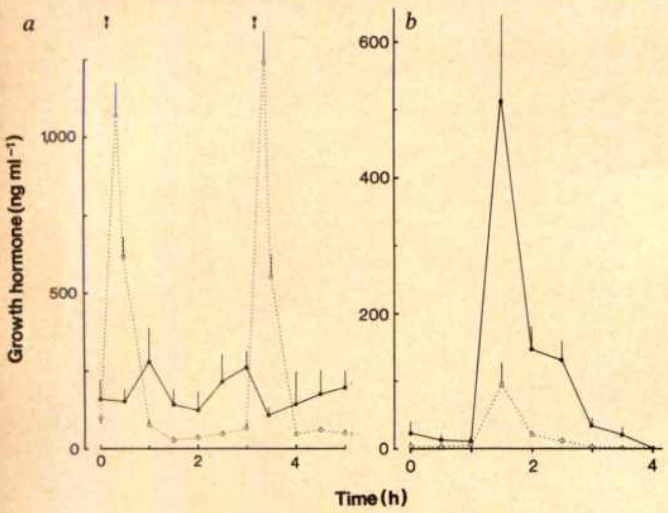


Fig. 1 Effects of hGHRF(1-29)NH_2 (*a*) and monosodium glutamate (*b*) on plasma GH levels. *a*, Six normal female rats (dotted line) received 1-μg injections of hGHRF(1-29)NH_2 (Bachem, UK, or Kabi-Vitrum, Sweden) via the i.v. cannulae at 9 a.m., 12 a.m., 3 p.m. and 6 p.m. Before and after the third and fourth injections (arrows), blood samples (100 μl) were withdrawn and the plasma separated and frozen. A second group of four female rats were given injections of saline to serve as controls (solid line). *b*, Newborn male rat pups received five subcutaneous injections of MSG (4 mg per g body weight) every second day from birth. Littermate controls received 10% saline. When 8 weeks old, the animals were catheterized as in *a* for blood sampling. Five MSG-treated rats (open circles) showed endogenous GH pulses which were blunted compared with those occurring in four normal male rats (solid circles). As endogenous GH pulses are not synchronized with respect to clock time, the peak concentrations for each individual rat have been aligned in this figure.

Methods. Sprague–Dawley rats (150–180 g) were housed individually in metabolic cages in a light- and temperature-controlled room and fed a powdered diet and distilled water *ad libitum*. Animals were anaesthetized with halothane/N_2O/O_2, and a silicone rubber catheter was inserted into the right jugular vein and exteriorized dorsally. Daily weight and food intake measurements showed that animals quickly resumed their normal growth. Sequential blood samples were obtained from conscious animals via extension cannulae attached to the catheters. Plasma GH levels were determined by RIA on 5- and 20-μl plasma samples using reagents supplied by NIADDK.

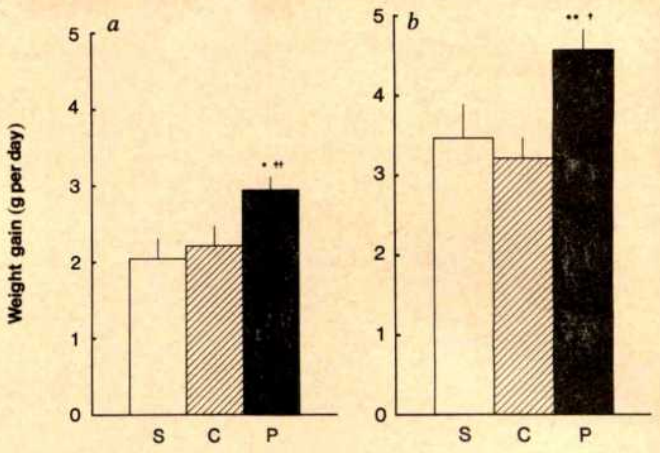


Fig. 2 Effects of continuous and pulsatile administration of hGHRF(1-29)NH_2 on growth in normal female (*a*) and MSG-treated male (*b*) rats. Values shown are means ± s.e.m. In both experiments, analysis of variance showed significant differences between the three treatment groups (P, pulsatile hGHRF (1-29)NH_2, solid bars; C, continuous hGHRF(1-29)NH_2, hatched bars; S, saline infused controls, open bars). In female animals (*a*), pulsatile GHRF produced significantly greater weight gains than either continuous GHRF ($^*P < 0.05$) or saline treatment ($\dagger\dagger P < 0.025$). In MSG-treated male rats (*b*), pulsatile GHRF again was most effective ($\dagger P < 0.05$ vs. saline, and $^{**}P < 0.005$ vs. continuous GHRF).

Methods. Sprague–Dawley rats (150–180 g) were anaesthetized, a silicone cannula was placed in the right jugular vein, a head plate screwed to the occipital bone and the cannula passed through a protective spring to a fluid-tight swivel joint connected to multichannel peristaltic infusion pumps[11,12]. One pump ran continuously (1.6 ml per day) whereas a second pump delivered the same daily volume as a series of 200-μl pulses (2 min duration, every 3 h). The vehicle was phosphate-buffered saline (Dulbecco's 'A') containing 0.01% rat albumin and heparin (5 U ml^{-1}). The animals were weighed daily, which entailed disconnecting the continuous infusion for less than 2 min. Growth rates (g per day) were calculated by regressing the increase in body weight against time for each animal.

blood samples from three of the rats 5, 10 and 20 min after the 50th pulse of hGHRF(1-29)NH_2; the plasma GH values (mean ± s.e.m.) were 3,595 ± 1,400, 910 ± 155 and 326 ± 68 ng ml^{-1}, respectively. Pulsatile GHRF treatment produced a 30% increase in body weight gain compared with saline-infused controls, whereas the same total daily dose of hGHRF (1-29)NH_2 given as a continuous infusion for 12 days had no significant effect (Fig. 2*a*). A similar stimulating effect of pulsatile hGHRF(1-29)NH_2 on proximal tibial growth[13] was observed in the same groups of animals (controls 110 ± 5; continuous GHRF 103 ± 8; pulsatile GHRF 117 ± 2 μm per day, mean ± s.e.m.), although the difference between continuous and pulsatile GHRF treatment was less clearly established ($P = 0.056$). At the end of the experiment, the anterior pituitary glands were extracted and assayed for GH content (Table 1*a*). Pulsatile hGHRF(1-29)NH_2 treatment significantly increased the pituitary content of GH, whereas continuous infusions were without effect.

We also compared continuous and pulsatile administration of hGHRF(1-29)NH_2 in animals made deficient in endogenous GHRF by neonatal exposure to monosodium glutamate (MSG)[14]. This treatment produces stunted growth[15] and blunts endogenous GH pulses (Fig. 1*b*). However, MSG-treated rats can secrete large amounts of GH in response to exogenous GHRF. In five conscious MSG-treated male rats implanted with indwelling jugular cannulae, plasma GH values rose from 33 ± 7 ng ml^{-1} to 2,640 ± 1,070 and 131 ± 26 ng ml^{-1}, respectively, 10 and 30 min after a single i.v. injection of 1 μg hGHRF (1-29)NH_2. Using the programmable i.v. infusion system, groups of MSG-treated male rats were given saline or hGHRF (1-29)NH_2, either continuously (8 μg per day) or in a pulsatile pattern (1-μg pulses every 3 h) for 10 days. As in normal female rats, pulsatile hGHRF(1-29)NH_2 stimulated body weight gain whereas continuous infusions of this peptide did not increase weight gain (Fig. 2*b*). Pulsatile hGHRF(1-29)NH_2 also produced a significant increase in long bone and tail growth in these MSG-treated rats (Table 2).

Although neonatal exposure to MSG results in smaller anterior pituitary glands and lower GH content in the adult, the pituitary GH concentration remains unchanged[16]. Unlike in normal female rats, we could show no trophic effects of hGHRF(1-29)NH_2 on pituitary GH content in MSG-treated animals (Table 1*b*). GHRF can exert a trophic effect *in vitro* on GH messenger RNA transcription[17] in addition to its powerful secretagogue effect. However, multiple injections of GHRF do not increase the GH content of pituitary tissue autotransplanted to the kidney capsule[18]. As MSG disrupts other hypothalamic neurotransmitter systems[19] in addition to GHRF neurones[14], it is possible that the trophic effect of GHRF *in vivo* requires an intact hypothalamo-hypophysial system.

These experiments are the first to show that a GHRF peptide can induce growth in both normal female and GHRF-deficient animals, and that the amount of growth depends on the pattern of administration. The female rat has been relatively neglected in studies of GHRF *in vivo* and offers considerable advantages

Table 1 Effect of hGHRF(1-29)NH_2 on anterior pituitary stores of GH

	(*n*)	Wet weight (mg)	Anterior pituitary GH content (μg)	GH concentration (μg per mg wet wt)
a Normal females				
Saline	(8)	9.8 ± 0.3	613 ± 36	62 ± 3
Continuous GHRF	(8)	10.2 ± 0.7	623 ± 68	61 ± 5
Pulsatile GHRF	(8)	10.3 ± 0.4	834 ± 75*	81 ± 7*
b MSG-treated males				
Saline	(8)	4.5 ± 0.5	522 ± 119	104 ± 17
Continuous GHRF	(8)	4.6 ± 0.5	577 ± 112	115 ± 14
Pulsatile GHRF	(8)	5.3 ± 0.6	680 ± 161	115 ± 20

Anterior pituitary glands were removed, weighed, homogenized individually in 1 ml phosphate-buffered saline *p*H 7.4, centrifuged for 10 min at 13,000*g* and the supernatants assayed for rat GH by radioimmunoassay (RIA), using six doubling dilutions in duplicate. See text for an explanation of the different treatment groups. In female rats (*a*), pulsatile hGHRF(1-29)NH_2 for 12 days increased pituitary GH content and concentration. In male MSG-treated rats (*b*) hGHRF(1-29)NH_2 had no significant effect on pituitary GH.
* $P < 0.05$ vs. both saline and continuous GHRF.

of uniformity and availability, together with an intact hypothalamo-hypophysial system. The possibility that prolonged pulsatile GHRF treatment might alter reproductive cycles in the female rat, and thus indirectly influence body growth must be considered, although this explanation is unlikely to account for the stimulation of growth produced in male rats by pulsatile GHRF. The simplest explanation for our results is that effective GHRF levels in the hypophysial portal vessels are achieved only by giving the peptide in pulses. Continuous infusions of GHRF require higher total dose rates to stimulate GH release and can result in rapid depletion of pituitary GH stores[20]. In the case of MSG-treated animals, we are replacing the depleted GHRF secretion, whereas in normal female rats we are presumably overriding endogenous GHRF secretion. Whether or not the endogenous secretory profile of GHRF in male rats shows 3-hourly episodes, an exogenous GHRF peptide given in 3-hourly pulses produces a plasma GH profile which mimics the normal male pattern. We have previously shown in hypophysectomized male rats that the growth responses to a given dose of GH are greater if the GH is given in 3-hourly pulses[12]. The pattern of GH secretion is less pulsatile in female rats, and this may partly underlie their slower growth rates[21]. We reasoned that maintaining a male pattern of GH secretion might increase the growth rate of normal females towards that of the male rat. This proved to be the case.

Both normal female rats and MSG-treated male rats may provide useful data when considering the treatment of GHRF-deficient children with pulsatile GHRF[7], especially where this has to be given by the subcutaneous route, which inevitably reduces the pulsatility. Our demonstration that a more effective GH secretory profile can be imposed in normal, intact (female) animals by patterned exogenous GHRF may well have applicability in animal husbandry, provided suitable simple means for achieving the optimal pattern of administration can be found. The obvious corollary of this result is that a sub-optimal infusion regime might impose a less effective growth rate, which could conceivably be useful therapeutically in children with excessive rates of growth. At its simplest level, the chronically cannulated female rat offers a convenient model in which to test the effects of different routes and patterns of administration of GHRF, its analogues and inhibitors on whole body growth.

Table 2 Effect of hGHRF(1-29)NH_2 on tail and bone growth in MSG-treated rats

	(*n*)	Tail growth (mm)	Proximal tibial growth (μm per day)
Saline	(8)	15.4 ± 1.3	137 ± 12
Continuous	(8)	18.2 ± 2.6	137 ± 8
Pulsatile	(8)	20.6 ± 1.3*	159 ± 7†

Tail lengths of MSG-treated rats were measured[22] under anaesthesia at the beginning and end of the infusion experiment (see text). The differences between these measurements are shown as mean ± s.e.m. Immediately before the infusions began, each rat received an injection of oxytetracycline (2 mg per 100 g body weight, i.v.). At the end of the experiment, the proximal tibias were dissected out and cumulative bone growth assessed by fluorescence microscopy according to the method of Hansson *et al.*[13]. Results were analysed using paired *t*-tests against littermate controls.
* $P < 0.001$ vs. saline; † $P < 0.05$ vs. continuous GHRF.

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1. Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300**, 276-278 (1982).
2. Guillemin, R. *et al. Science* **218**, 585-587 (1982).
3. Ling, N. *et al. Proc. natn. acad. Sci. U.S.A.* **81**, 4302-4306 (1984).
4. Wehrenberg, W. B. *et al. Biochem. biophys. Res. Commun.* **109**, 382-387 (1982).
5. Wehrenberg, W. B. *et al. Endocrinology* **111**, 2147-2148 (1982).
6. Wehrenberg, W. B. *et al. Neuroendocrinology* **36**, 489-491 (1983).
7. Thorner, M. O. *et al.* in *Proc 7th Int. Congr. Endocr.* Abstr S. 167 (Excerpta Medica, Amsterdam, 1984).
8. Wehrenberg, W. B., Baird, A. & Ling, N. *Science* **221**, 556-558 (1983).
9. Tannenbaum, G. S. & Martin, J. B. *Endocrinology* **98**, 562-570 (1976).
10. Eden, S. *Endocrinology* **105**, 555-560 (1979).
11. Clark, R. G. & Robinson, I. C. A. F. *J. Physiol., Lond.* **350**, 2P (1984).
12. Clark, R. G., Jansson, J. O., Isaksson, O. & Robinson, I. C. A. F. *J. Endocr.* **104**, 53-61 (1985).
13. Hansson, L. I., Menander-Sellman, K., Strenstrom, A. & Thorngren, K.-G. *Calcif. Tissue Res.* **10**, 238-251 (1972).
14. Bloch, B. *et al. Nature* **307**, 272-273 (1984).
15. Olney, J. W. *Science* **164**, 719-721 (1969).
16. Millard, W. J., Martin, J. B. Jr, Audet, J., Sagar, S. M. & Martin, J. B. *Endocrinology* **110**, 540-550 (1982).
17. Barinaga, M. *et al. Nature* **306**, 84-86 (1983).
18. Jansson, J. O., Carlsson, L. & Isaksson, O. *Endocrinology* (in the press).
19. Jennes, L., Stumpf, W. E., Bissette, G. & Nemeroff, C. B. *Brain Res.* **308**, 245-253 (1984).
20. Wehrenberg, W. B., Brazeau, P., Ling, N., Textor, G. & Guillemin, R. *Endocrinology* **114**, 1613-1616 (1984).
21. Jansson, J. O., Ekberg, S., Isaksson, O. & Eden, S. *Endocrinology* **114**, 1287-1294 (1984).
22. Hughes, P. C. R. & Tanner, J. M. *J. Anat.* **106**, 349-370 (1970).

Tissue-specific expression of rat myosin light-chain 2 gene in transgenic mice

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One approach to determining how the differential expression of specific genes is regulated in higher organisms is to introduce cloned copies of the genes (or parts of the genes) into the genomes of individual organisms from the very beginning of their development. The way in which the exogenous genetic information behaves during the development of the experimental organisms can then provide a means of defining the DNA sequences that restrict the

expression of the gene to specific cell types and times of development. So far, several different genes have been introduced into the genomes of mice[1–15], but in only a few cases have the exogenous genes retained the tissue specificity of expression of the equivalent endogenous genes[12,14,15]. I report here that in two out of three 'transgenic' mice carrying copies of the rat gene for skeletal muscle myosin light chain 2, the exogenous gene is expressed specifically in skeletal muscle cells. The sequences contained in the cloned copy of the myosin light-chain 2 gene used in these experiments are thus sufficient to confer a tissue-specific pattern of expression.

A recombinant plasmid (pRH3MLC)[16] containing the rat skeletal muscle myosin light-chain 2 (MLC2) DNA sequences in their entirety, in a 4.7-kilobase (kb) DNA fragment, was cut with *Hin*dIII to release the rat DNA sequences from the plasmid DNA and used for injection into fertilized mouse eggs (Fig. 1*a*). Superovulated females of (BALB/c×C57BL/6J) F_1 were mated to males of (C57BL/6J×DBA) F_1. On the day after mating, fertilized eggs were recovered from the oviducts. The male pronuclei were microinjected with 1–2 pl containing ~500 molecules of the plasmid DNA. Eggs that survived the microinjection (~50%) were implanted into the oviducts of pseudopregnant CD-1 females for further development. Spleen DNA samples from 26 neonatal mice were analysed by Southern blot hybridization and four mice containing pRH3MLC sequences were identified. DNA isolated from spleens of these mice (designated MLC_1, MLC_2, MLC_3, MLC_4) and a control mouse was digested with *Hin*dIII, *Eco*RI or *Xba*I. Figure 1*b* shows an autoradiogram of a Southern blot of the fractionated DNA, hybridized to ^{32}P-labelled pRH3MLC DNA. In addition to the expected 4.7- and 4.3-kb DNA fragments in the *Hin*dIII digest, several minor fragments that hybridized to the probe could be observed in DNA from mice MLC_1, MLC_2 and MLC_4, these fragments resulting from either junction fragments with cellular DNA or rearranged sequences.

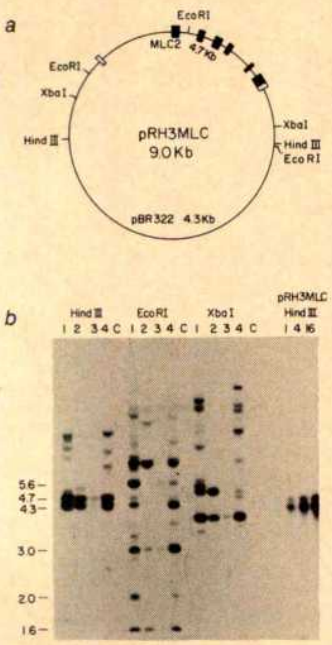


Fig. 1 Map of plasmid DNA pRH3MLC and Southern blot analysis of DNA from four transgenic mice. *a*, The 4.7-kb *Hin*dIII DNA fragment containing the rat MLC2 gene was cloned into the *Hin*dIII site of pBR322 (ref. 16). Boxes indicate the positions of the seven exons; empty boxes represent the 5′- and 3′-untranslated regions; solid boxes represent coding sequences. *b*, Total spleen DNA (10 μg) from the four transgenic mice (1–4) and a control mouse DNA (C) was digested with *Hin*dIII, *Eco*RI or *Xba*I. The DNA fragments were fractionated on a 0.8% agarose gel, transferred to nitrocellulose filter and hybridizes with ^{32}P-labelled pRH3MLC DNA. The blot was washed in stringent conditions (0.1×SSC, 0.2% SDS at 75 °C for 2 h) not allowing detection of the mouse gene. Plasmid pRH3MLC DNA samples digested with *Hin*dIII corresponding to 1, 4 and 16 copies per diploid mouse genome were included to estimate the copy number of inserted DNA in transgenic mice. Molecular sizes are shown in kb on the left.

As the pRH3MLC DNA was cut with *Hin*dIII before microinjection, multiple orientations of MLC2 and pBR322 DNA sequences were expected (Fig. 1). For example, in the *Eco*RI digests, the 1.6-kb DNA fragment seen in the four transgenic mice is a product of cleavage at the internal *Eco*RI sites of the MLC2 gene. The 2.0-kb DNA fragment in transgenic mice MLC_1, MLC_3 and MLC_4 is a product of cleavage at the 3′ end *Eco*RI site of MLC2 gene, when it is integrated next to pBR322 DNA in a head-to-tail configuration. This fragment is undetectable in mouse MLC_2 DNA; instead a predominant 6.3-kb DNA fragment appears, indicating that most of the MLC2 DNA molecules in this mouse are arranged in tandem arrays with pBR322 DNA in a head-to-head orientation. Similarly, in the *Xba*I digests, the 3.9-kb DNA fragment is a product of cleavage at the two sites when pBR322 DNA is flanked on both sides with MLC2 DNA sequences. Analysis of the restriction pattern indicated that most of the copies of the injected DNA are integrated tandemly in the mouse genome. The number of pRH3MLC DNA copies in each mouse was estimated by comparing the intensity of the hybridized fragments with those of known amounts of plasmid DNA. Thus, mice MLC_1, MLC_2, MLC_3 and MLC_4 contain ~20, 10, <1 and 16 copies per cell, respectively.

To examine the transmission of the inserted DNA sequences to progeny, each of the four transgenic mice was mated with a normal mouse. Southern blot hybridization to spleen DNA from the progeny of MLC_1, MLC_2 and MLC_4 revealed that the inserted DNA sequences were transmitted faithfully through the germ line to ~50% of the progeny (data not shown; see Fig. 2), the expected frequency if the majority of the multiple MLC2 copies are integrated tandemly at a single site of one chromosome.

In contrast to the mendelian inheritance of pRH3MLC DNA sequences in mice MLC_1, MLC_2 and MLC_4, mouse MLC_3 did not transmit the injected DNA to any of its first 60 offspring. This female mouse contains <1 copy per cell of the inserted DNA (Fig. 1*b*) and is apparently a mosaic in which the injected DNA is present in only a small proportion of its somatic cells.

To investigate whether the expression of the rat MLC2 gene in transgenic mice was tissue-specific, RNA was extracted from skeletal muscle and other organs from the progeny of two transgenic mice. The presence of rat MLC2 transcripts in these RNA preparations was determined by the S_1 endonuclease technique, using as a probe the coding strand of the 630-base pair (bp) *Hin*fI/*Hin*fI DNA fragment derived from the 5′ region of the rat MLC2 gene and labelled at the 3′ end with reverse transcriptase (see Fig. 3). In stringent hybridization conditions

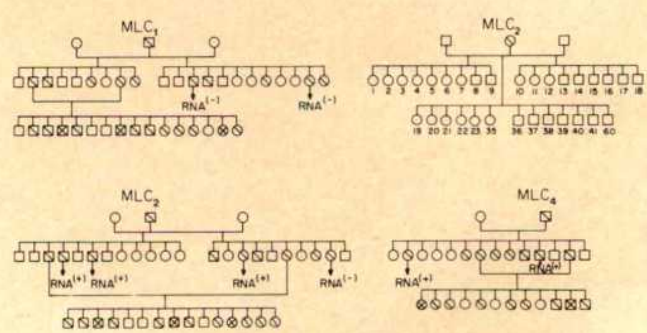


Fig. 2 Inheritance of pRH3MLC sequences in the four transgenic mice. Each transgenic mouse was mated with normal mouse Heterozygous male and female progeny of MLC_1 and MLC_2 mice were mated to give rise to normal homozygous mice. Mouse MLC_3 is probably a mosaic. Squares represent males, circles females, intersecting diagonals homozygous mice, single diagonals heterozygous mice. Mice denoted $RNA^{(+)}$ contained MLC2 transcripts in skeletal muscle whereas mice denoted $RNA^{(-)}$ did not.

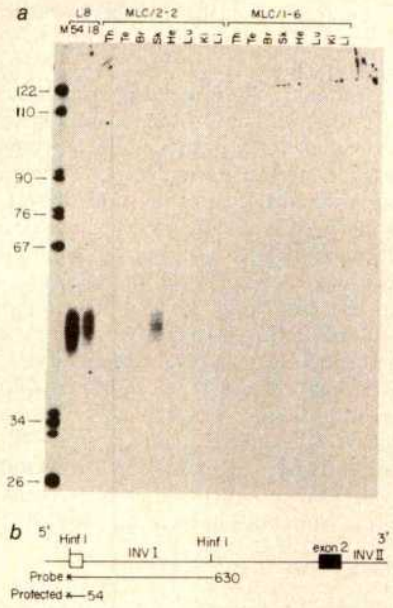


Fig. 3 S_1 analysis of rat MLC2 RNA in the progeny of transgenic mice MLC_1 and MLC_2. *a*, 25 μg total RNA from the indicated tissues and total RNA from differentiated cultures of the myogenic cell line L8 were hybridizied at 46 °C for 16 h to a 3′-end-labelled *Hin*fI/*Hin*fI DNA fragment prepared from pRH3MLC DNA (see scheme in *b*). Th, thymus; Te, testes; Br, brain; Sk, skeletal muscle; He, cardiac muscle; Lu, lung; Ki, kidney; Li, liver. Hybridization reactions were incubated with 5,000 U S_1 nuclease (Miles) for 30 min at 42 °C. S_1-resistant hybrids were sized on 5% acrylamide/7M urea gel. *b*, Map of the 5′ region of the rat MLC2 gene and the hybridization probe. Boxes, exons; solid lines, introns and 5′-flanking region; open box, 5′-untranslated exon; solid box, first coding exon. The 3′ label is indicated by an asterisk and the lengths of the probe and the protected fragment are also indicated.

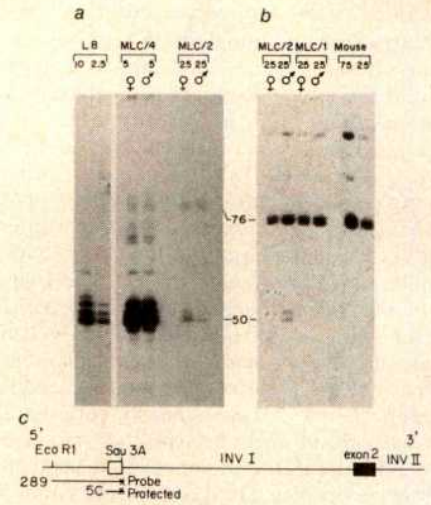


Fig. 4 The rat MLC2 gene is initiatied correctly in progeny of transgenic mice MLC_2 and MLC_4. Total RNA from skeletal muscle of male (♂) and female (♀) progeny of transgenic mice MLC_1, MLC_2 and MLC_4, RNA from skeletal muscle of control mouse and from differentiated cultures of the myogenic cell line L8 were hybridized with 5′-end-labelled *Sau*3A/*Eco*RI DNA fragment (see *c*). RNA (μg) in each hybridization reaction is indicated. *a*, Hybridization performed at 42 °C and S_1 digestion at 37 °C; *b*, hybridization at 37 °C and S_1 digestion at 32 °C. After treatment with S_1 nuclease, the resistant hybrids were sized on 5% acrylamide/7 M urea gels. *c*, Map of the 5′ region of the rat MLC2 gene and the probe used in the hybridization. Boxes, exons; solid line, introns and 5′-flanking region; open box, 5′-untranslated exon; solid box, first coding exon. The 5′ label is indicated by an asterisk and the lengths of the probe and the protected fragment are shown.

(hybridization at 46 °C and S_1 digestion at 42 °C), cross-hybridization to the endogenous mouse MLC2 transcripts did not occur. Authentic rat transcripts protect a 54-nucleotide DNA fragment from S_1 digestion. The appearance of several bands at that region probably results from S_1 nibbling at the end. Figure 3 shows that no rat MLC2 transcripts could be detected in skeletal muslce RNA or any of the other seven tissues of mouse MLC_1. In mouse MLC_2, however, transcripts were detected in skeletal muscle RNA but not in RNA from the other tissues tested. Analysis of RNA prepared from mouse MLC_4 revealed the presence of a large amount of the rat transcripts only in skeletal muscle (data not shown).

Muscle-specific transcripts in transgenic mice MLC_2 and MLC_4 could arise either from readthrough transcription initiated from upstream mouse promoter expressed in skeletal muscle or from initiation at the authentic rat MLC2 promoter. To distinguish between these two possibilities, the site of the initiation of these transcripts was determined. The coding strand of a 289-bp *Sau*3A/*Eco*RI DNA fragment ^{32}P-labelled with T4 polynucleotide kinase was hybridized to RNA from differentiated cultures of the rat myogenic cell line L8, to control mouse skeletal muscle RNA and to skeletal muscle RNA from males and females of the first generation progeny of mice MLC_1, MLC_2 and MLC_4. RNA from the rat myogenic cell line protected a 50-nucleotide DNA fragment (Fig. 4). The appearance of two bands spaced one nucleotide apart probably results from S_1 nibbling of the sequence that immediately precedes the cap site. RNA from control mouse skeletal muscle protected a 76-nucleotide DNA fragment. These unexpected results suggest that: (1) there is a similarity in the sequences of the 5′-untranslated exon and immediate flanking region of the rat and the mouse MLC2 genes and (2) the transcription of the mouse MLC2 gene is initiated ~20 nucleotides 5′ to the initiation site of the rat MLC2 gene. In more stringent hybridization conditions, such as those chosen for the *Hin*fI DNA fragment (Fig. 3), there was no cross-homology with the mouse exon. (Also, compare the intensity of the signals of the 76-nucleotide band in Fig. 4*a*, *b*.) The stringency was lowered here so that the hybridization to the mouse exon is an internal control for the intactness of the RNA preparations of mouse MLC_1 and the control mouse. RNA from three out of four progeny of transgenic mouse MLC_2 and two progeny of mouse MLC_4 protected the diagnostic 50-nucleotide DNA fragment (Fig. 4). As expected, skeletal muscle RNA of MLC_1 mice gave no detectable signal.

The MLC2 gene is a member of a large group of genes activated during terminal differentiation of muscle cells[17]. The results presented here demonstrate that in two out of the three transgenic mice tested, the injected rat MLC2 gene responded to normal developmental controls. Its expression was detected only in skeletal muscle. Thus, the 4.7-kb rat DNA fragment containing the entire MLC2 gene includes *cis*-acting sequences sufficient to specify the correct expression of this gene. RNA transcripts of this gene were found in skeletal muscle in three of four progeny of MLC_2 and in two progeny of MLC_4, and not in any other tissue. The RNA had the correct initiation site of the authentic rat MLC2 gene, which differs from the endogenous mouse MLC2 by ~26 nucleotides. Interestingly, a similar frequency of non-expressing progeny (two of eight) has been reported for transgenic mice expressing the rabbit β-globin gene in skeletal muscle[1].

The levels of expression of the inserted rat gene are ~5–10% (in MLC_2) and 5–10× greater (in MLC_4) than in differentiated cultures of the rat myogenic cell line L8. The variable levels of specific expression could result from position effect, thus integration into non-homologous chromosomal sites may influence the level of expression of the foreign gene.

Several genes have been inserted into mice[1–15]. Tissue-specific expression of introduced genes, however, has been reported

only in four other cases; the rearranged immunoglobulin in κ gene[12], the rearranged immunoglobulin heavy-chain gene[15], the rat pancreatic elastase I gene[14] and the fused gene consisting of the 5′ end of the mouse β-globin gene spliced to the 3′ end of the human β-globin gene (F. D. Constantini, personal communication). In the first three cases and in the rat MLC2 gene in mouse MLC_4, the level of expression was similar to that of the endogenous gene, whereas in the globin and rat MLC2 genes in mouse MLC_2, the level of expression was considerably lower than the endogenous counterparts. One possible interpretation is that the immunoglobulin and the elastase I genes include tissue-specific enhancer sequences that are recognized as such even when the genes are integrated at different chromosomal sites. Evidence for the dramatic effects of enhancer sequences on the efficiency of expression of genes introduced into somatic cells has been obtained[18]. There is no evidence that the injected MLC2 DNA fragment includes specific enhancer elements. Therefore, the hundredfold difference in the level of expression in the two transgenic mice could result from either the absence of such elements or their weakness. Thus, in mouse MLC_2, these sequences are neutralized easily by the local chromosomal environment at the site of integration, whereas in mouse MLC_4 they overcome the position effect.

Production of transgenic mice expressing muscle-specific genes or specifically modified genes in the appropriate tissue would allow a detailed study of the parameters affecting tissue-specific gene expression (for example, site of integration, DNA methylation or chromatin organization). Moreover, the control of expression of the inserted genes can now be analysed in myogenic and non-myogenic cells isolated from these mice.

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1. Lacy, E., Roberts, S., Evans, E. P., Burtenshaw, M. D. & Costantini, F. D. *Cell* **34**, 343–358 (1983).
2. Gordon, J. W. & Ruddle, F. H. *Science* **213**, 1244–1246 (1981).
3. Wagner, E., Stewart, T. & Mintz, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5016–5020 (1981).
4. Wagner, T. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 6376–6380 (1981).
5. Costantini, F. & Lacy, E. *Nature* **294**, 92–94 (1981).
6. Brinster, R. L. *et al. Cell* **27**, 223–231 (1981).
7. Jahner, D. *et al. Nature* **298**, 623–628 (1982).
8. Palmiter, R. D. *et al. Nature* **300**, 611–615 (1982).
9. Burki, K. & Ullrich, A. *EMBO J.* **1**, 127–131 (1982).
10. Palmiter, R. D., Chen, H. Y. & Brinster, R. L. *Cell* **29**, 701–716 (1982).
11. McKnight, G. S., Hammer, R. E., Kuenzel, E. A. & Brinster, R. L. *Cell* **34**, 335–341 (1983).
12. Brinster, R. L. *et al. Nature* **306**, 332–336 (1983).
13. Stewart, T. A., Pattengale, P. K. & Leder, P. *Cell* **38**, 627–637 (1984).
14. Swift, C. H., Hammer, R. E., MacDonald., R. J. & Brinster, R. L. *Cell* **38**, 639–646 (1984).
15. Grosschedl, R., Weaver, D., Baltimore, D. & Costantini, F. *Cell* **38**, 647–658 (1984).
16. Nudel, U., Calvo, J., Shani, M. & Levy, Z. *Nucleic. Acids Res.* **12**, 7175–7186 (1984).
17. Carmon, Y., Czosnek, H., Nudel, U., Shani, M. & Yaffe, D. *Nucleic Acids Res.* **10**, 3085–3097 (1982).
18. Gruss, P. *DNA* **3**, 1–5 (1984).

Regulation of a collagen gene promoter by the product of viral *mos* oncogene

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Oncogenic transformation of cells produces important changes in the biosynthetic pattern of certain cellular proteins[1–6]. For example, the synthesis of type I collagen in transformed fibroblasts is severely reduced as a result of changes in transcription[7–10]. Here we report the results of DNA-mediated transfection experiments using recombinant plasmids in which the promoter region of the α2(I) collagen gene is fused to an easily recognizable marker gene, and cell lines expressing the marker gene are isolated. Our data show that the expression of the marker gene fused to the cloned α2(I) collagen promoter is strongly inhibited by v-*mos* transformation, suggesting that a common mechanism inhibits both the transfected and endogeneous α2(I) collagen promoters.

The α2(I) collagen gene is very large (~40 kilobase pairs(bp) with ~50 exons)[11] and is therefore difficult to manipulate *in vitro*. To overcome this problem in studying its expression by DNA-mediated transfection experiments, we have fused the promoter region of this gene to either the bacterial gene for chloramphenicol acetyltransferase (*cat*)[12] or the gene for aminoglycoside phosphotransferase (*neo*)[13]. Although such constructions may not contain all the regulatory elements controlling the expression of the endogenous α2(I) collagen gene, they do allow us to test whether the 5′-flanking sequences of the α2(I) collagen gene which are present in these hybrid plasmids are sufficient for regulation by oncogenes. Figure 1 shows the plasmids constructed for our experiments. We first transfected NIH 3T3 cells with DNA of plasmid pHO1000, in which the chick α2(I) collagen promoter is fused to the *cat* gene. This plasmid also contains the gene for guanine phosphoribosyltransferase (*gpt*) fused to the early promoter of simian virus 40 (SV40). Clones positive for the expression of both the *gpt* and *cat* genes (AT1, AT2, AT3, etc.) were isolated. One of these clones (AT3) was examined by Southern blotting to show that the plasmid was stably integrated throughout at least two cycles of subcloning. We estimated that this cell line contains about eight copies of the α2(I) collagen promoter-*cat* DNA unit (data not shown). A Northern hybridization experiment indicated that the RNA produced by the collagen promoter-*cat* transcription unit in these cells was a discrete species ~1,600 nucleotides (Fig. 2*a*), consistent with a transcript starting from within the cloned chicken α2(I) collagen promoter and ending at the SV40 polyadenylation site present downstream from the *cat* gene in pHO1000. The results of a primer extension experiment (Fig. 2*b*) further indicate that the *cat* gene transcript starts at the correct site in the cloned α2(I) collagen promoter. In the same cells, *gpt* transcripts initiate at the major transcription start site in the early SV40 promoter (Fig. 2*b*, lane 2).

To determine whether the expression of the hybrid α2(I) collagen promoter-*cat* transcription unit was also inhibited by oncogenic transformation after it had been introduced into NIH 3T3, we first transformed three cell lines with the mouse Moloney sarcoma and leukaemia virus complex (MMSV). In all three clones, the level of CAT activity was reduced 7–14-fold after transformation (Fig. 3*a*, Table 1*a*). Dot-blot hybridizations using RNA extracted from one of these lines showed that the levels of endogenous α2(I) collagen RNA and those of *cat* RNA decreased 8–10-fold after MMSV transformation, whereas the levels of actin RNA were unchanged (Fig. 3*b*).

The plasmid construction used in these experiments also harbours the early promoter of SV40, which is fused to the bacterial *gpt* gene (Fig. 1*a*); this SV40 promoter segment includes the SV40 enhancer sequences[14,15]. To determine whether SV40 enhancer sequences are required for inhibition of the α2(I) collagen promoter after MMSV transformation, NIH 3T3 cells were transfected with pAZ1005, a plasmid in which a mouse α2(I) collagen promoter segment is fused to the *neo* gene but which does not contain SV40 enhancer sequences (Fig. 1*c*). Several colonies resistant to the antibiotic G418 (positive for the expression of the *neo* gene) were isolated and transformed either by MMSV or by a plasmid containing the

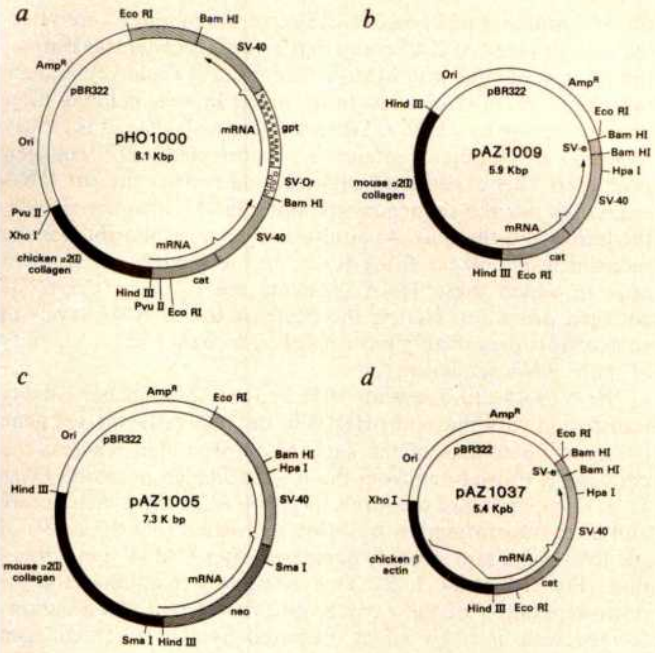


Fig. 1 Schematic representation of the recombinant plasmids used in the present work: pHO1000 (*a*), pAZ1009 (*b*), pAZ1005 (*c*) and pAZ1037 (*d*). *a*, pHO1000: This plasmid was constructed by H. Ohkubo (H. Ohkubo, in preparation). It contains a fragment of the chicken $\alpha 2$(I) gene which spans the chicken promoter region from 1,300 bp upstream of the start site of transcription to 110 bp downstream of this site[16]. This segment is fused to the gene for chloramphenicol acetyltransferase (*cat*) by means of *Hin*dIII linkers. The sequence 110 bp downstream of the start site of transcription of the $\alpha 2$(I) collagen gene does not contain the starting AUG for the collagen polypeptide. The SV40 small-T intron and the SV40 early region polyadenylation site contribute signals for RNA processing. This plasmid harbours a second transcription unit in which the early promoter of SV40 is fused to the gene for guanine phosphoribosyl transferase (*gpt*); this unit is inserted into the *Bam*HI site. This segment, which includes the SV40 small-T splice sites and intron as well as the SV40 early region poly(A) site, was isolated from pSV2-gpt[17]. *b*, pAZ1009: a *Mst*II/*Sph*I fragment of the mouse $\alpha 2$(I) collagen gene taken from plasmid pAZ1001 (ref. 18) was inserted by means of *Hin*dIII linkers into the *Hin*dIII site of the plasmid pSV0-cat (a derivative of pSV2-cat without the origin of replication and the early promoter of SV40, given by C. Gorman). This fragment contains 2,000 bp upstream of the start of transcription of the mouse $\alpha 2$(I) collagen gene (including the TATA box and CAT box) and 54 bp downstream of this site. These 54 bp do not contain the starting AUG of the $\alpha 2$(I) collagen peptide. The 3′ end of this transcription unit is composed of the SV40 small-T splice sites, the small-T intron and the SV40 early region polyadenylation site. The 884-bp *Hpa*I/*Eco*RI fragment was then replaced with a *Hpa*I/*Eco*RI fragment isolated from pPH2-a*Bam*R (a gift from S. Sarragosti). This fragment contains an SV40 segment between +113 and +270 in the SV40 map and includes one complete copy of the 72-bp enhancer and a second copy which is almost complete. The *Bam*HI and *Eco*RI restriction sites are contiguous to each other. *c*, pAZ1005: The same *Mst*II/*Sph*I fragment of the mouse $\alpha 2$(I) collagen gene as described in *b* was inserted by means of *Hin*dIII linkers into the *Hin*dIII site of the plasmid pSV0-neo (a derivative of pSV2-neo without the origin of replication and the early promoter of SV40, gift of C. Gorman). *d*, pAZ1037: The 1,350-bp *Xho*I/*Hin*cII fragment of the chicken β-cytoplasmic actin gene[19] was inserted in place of the mouse $\alpha 2$(I) collagen promoter region by means of *Xho*I and *Hin*dIII linkers. This fragment contains 350 bp preceding the start of transcription and 1,000 bp downstream of the transcription site, including the first exon, first intron and part of the second exon. The nucleotide sequences for the first 10 amino acids of β-actin are in-phase with the reading frame of the *cat* coding sequences. A similar gene fusion with a *Bacillus subtilis* fragment has been reported previously[20] in this case, although the CAT polypeptide became larger, the CAT specific activity was not affected.

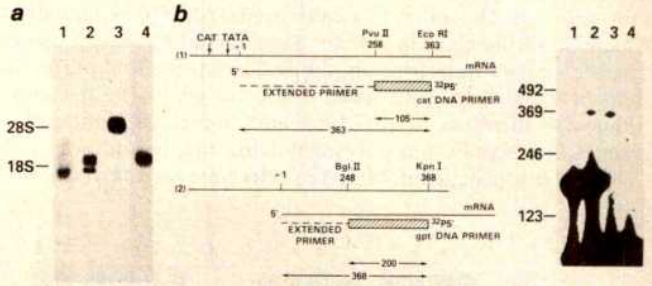


Fig. 2 Characterization of clone AT3-14, NIH 3T3 cells were transfected by the calcium phosphate method. Briefly, 3×10^5 NIH 3T3 cells grown in 10% calf serum were placed in a T-25 flask and 24 h later were transfected with 10 μg of pHO100 DNA. After a further 48 h, the cells were placed in *gpt* selection medium (25 μg ml^{-1} mycophenolic acid, 2 μM aminopterin, 2 μM thymidine, 10 μM hypoxanthine and 5 μg ml^{-1} xanthine) at 10^5 cells per 100-mm dishes. Colonies resistant to mycophenolic acid were isolated after 14 days and tested for CAT activity. ***a***, *cat* mRNA synthesis in clone AT3-14: total RNA was isolated from clone AT3 by the guanidine hydrochloride method[21]. RNA (10 μg) was fractionated by formaldehyde gel electrophoresis[22], blotted to nitrocellulose paper and hybridized to the following ^{32}P-labelled DNA probes as described elsewhere[18]: lane 1, 250 bp *Eco*RI/*Hin*dIII *cat* probe; lane 2, 350-bp *Kpn*I/*Hin*dIII *gpt* DNA; lane 3, 850-bp *Eco*RI/*Sma*I fragment of mouse $\alpha 2$(I) collagen DNA; lane 4, 1.6-kbp *Pst*I/*Pst*I fragment of a chicken β-actin cDNA clone[23] (a gift of Bruce Paterson). The positions of the 28S and 18S ribosomal RNAs are marked. ***b***, Start of transcription of *cat* and *gpt* mRNA: (1) The recombinant plasmid pAZ1009 was digested with *Eco*RI. The 5′ ends of this DNA were labelled by T4 polynucleotide kinase, then digested with *Pvu*I, generating a labelled 120-bp fragment. This fragment was used as primer and hybridized to poly(A)$^+$ RNA (16 μg) isolated from AT3-14 cells (lane 3) or to poly(A)$^+$ RNA (16 μg) of untransfected cells (lane 4) and then extended by reverse transcriptase[16]. (2) The recombinant plasmid pSV2-gpt was digested with *Kpn*I,5′-end labelled and digested with *Hin*dIII, generating a 200-bp fragment which was used as primer. The 5′-end-labelled primer was hybridized to poly(A)$^+$ RNA of clone AT3-14 (lane 2) or to poly(A)$^+$ RNA (16 μg) of untransfected NIH 3T3 cells (lane 1), and was extended by reverse transcriptase. The sizes of the resulting cDNAs were analysed by electrophoresis on a 7 M urea 5% polyacrylamide gel.

v-*mos* oncogene linked to the MMSV long terminal repeat sequence (pHT25, given by G. Van de Woude). The results (Fig. 3*c*) indicate that the levels of *neo* RNA decrease markedly in these cells after v-*mos* transformation. The presence of the SV40 enhancer sequences is therefore not required for inhibition of the transfected $\alpha 2$(I) collagen promoter.

In parallel with these experiments, we also used a plasmid in which the mouse $\alpha 2$(I) collagen promoter segment is fused to the *cat* gene. This plasmid, pAZ1009, also contains a small segment with the SV40 enhancer sequence (see Fig. 1*b*). NIH 3T3 cells were co-transfected with pAZ1009 together with pSV2-neo, a plasmid in which the *neo* gene is linked to the early SV40 promoter. Several G418-resistant colonies were isolated and transformed by MMSV. Table 1*b* shows that the levels of CAT are decreased in four of the five transformed cell lines examined, although the degree of reduction varies between cell lines, perhaps as a result of the location of plasmid integration in the genome.

As a control for these experiments, we used cell lines that had been transfected with either one of two plasmids containing the chicken β-actin promoter. The first plasmid (pβ6 actin) contains the entire chick β-actin gene cloned in PUC-8 (a gift of B. Paterson). NIH 3T3 cells were co-transfected with pβ6 actin and pSV2-neo, and G418-resistant colonies were selected. Figure 4 shows that the levels of β-actin RNA correctly initiated from the transfected β-actin promoter are not reduced after MMSV transformation, but in the same cells, the levels of endogenous $\alpha 2$(I) collagen RNA are sharply decreased (data

not shown). The other plasmid used (pAZ1037) contains a fragment of the chicken β-actin gene, including 350 bp preceding the start of transcription, the first exon, the first intron and part of the second exon fused to the *cat* gene. The plasmid is otherwise identical to pAZ1009 and, therefore, contains the same splice signals and polyadenylation site, in addition to the SV40 enhancer segment. NIH 3T3 cells were co-transfected with this plasmid and pSV2-neo, G418-resistant colonies were selected and the levels of CAT compared before and after transformation of the colonies with MMSV. The results (Table 1*c*) indicate that the levels of CAT show little change in most colonies after transformation by MMSV. When a decrease occurs, it is clearly less than in colonies containing a transfected α2(I) collagen promoter. This experiment suggests that neither the *cat* DNA sequences nor the sequences spanning the SV40 splice signals, the intron and the poly(A) addition site are responsible for the reduced levels of *cat* RNA found in the MMSV-transformed cells in which these DNA elements are fused to the α2(I) collagen promoter. Hence, the decrease in *cat* RNA levels in such cells is presumably not caused by a change in the stability of these RNA sequences.

The AT3-14 cell line is an NIH 3T3 cell that has been stably transfected with plasmid pHO1000. In these cells, the *gpt* gene is under the control of the early SV40 promoter, whereas the *cat* gene is transcribed from the α2(I) collagen promoter (Figs 1, 2). Comparison of the levels of *gpt* RNA in these cells before and after transformation by MMSV revealed that the levels of *gpt* RNA were also strongly decreased after MMSV transformation (Fig. 3*d*, lanes 1, 2). One possible explanation for the down-regulation of the early SV40 promoter in MMSV-transformed cells is a *cis* effect mediated by the α2(I) collagen promoter present on the same plasmid. NIH 3T3 cells were, therefore, co-transfected with pSV2-cat and pSV2-neo, and G418-resistant colonies were isolated and transformed by

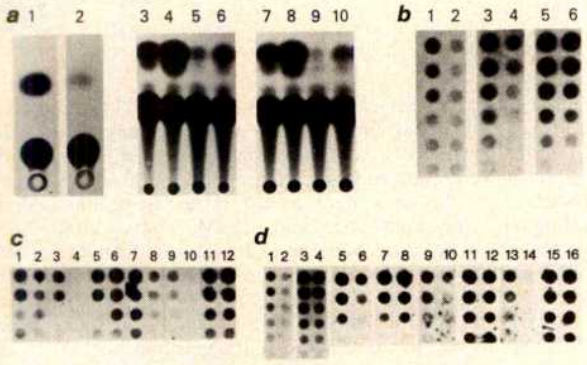


Fig. 3 Expression of the recombinant α2(I) collagen promoter (*a*–*c*) and of the recombinant SV40 early promoter (*d*) in normal and transformed cells. Clones of NIH 3T3 stably transfected with pHO1000 (*a*, *b*) or pAZ1005 (*c*) were withdrawn from the selection medium and transformed by a mixture of murine Moloney sarcoma virus and murine leukaemia virus (MMSV) or by transfection with the v-*mos* gene. *a*, Levels of CAT activity in normal and MMSV-transformed clones of NIH 3T3 cells stably transfected with pHO1000. 5×10^5 cells, normal (lanes 1, 3, 4, 7, 8) and transformed (lanes 2, 5, 6, 9, 10), were seeded in 100-mm dishes. After 48 h, the level of CAT was measured as described by Gorman *et al.*[12], except that equal amounts of proteins were used in the assays: lanes 1 and 2, clone AT3-14, 0.4 mg protein extract per assay; lanes 3–6, clone AT1; lanes 7–10, clone AT2; lanes 3, 5, 7, 9, 0.15 mg protein per assay; lanes 4, 6, 8, 10, 0.75 mg protein per assay. *b*, RNA dot blots from clone AT3-14. 1.5×10^6 AT3-14 cells (lanes 1, 3, 5) or MMSV-transformed AT3-14 cells (lanes 2, 4, 6) were seeded in 100-mm dishes. After 24 h, total RNA was extracted and twofold serial dilutions (in 20×SSC with 25 μg ml^{-1} bacterial rRNA) of total RNA (starting with 10 μg RNA per dot) were applied to nitrocellulose paper using a dot-blot template. The levels of α2(I) collagen RNA (lanes 1, 2), *cat* RNA (lanes 3, 4) and actin RNA (lanes 5, 6) were measured using the hybridization probes described in Fig. 2 legend. *c*, RNA dot-blot hybridization of clones of NIH 3T3 stably transfected with the plasmid pAZ1005. RNA was isolated and spotted for dot-blot hybridization as described in *b*. Lanes 1–6, clone AZ51; lanes 7–12, clone AZ57; lanes 1, 3, 5, 7, 9, 11 are of RNA from normal cells; lanes 2, 4, 6, 8, 10 and 12 are of RNA from v-*mos*-transformed cells; lanes 1, 2, 7 and 8 were hybridized with the same α2(I) collagen probe. Lanes 3, 4, 9 and 10 were hybridized with a *neo* probe consisting of a 1,300-bp *Sma*I/*Hin*dIII fragment of pAZ1005; lanes 5, 6, 11 and 12 were hybridized with the β-actin probe. *d*, Activity of the early SV40 promoter in normal and MMSV-transformed NIH 3T3 cells. Cells were stably transfected with plasmids pHO1000 (lanes 1–4, clone AT3-14) or pSV2-neo (lanes 5–8, clone SV7; lanes 9–12, clone SV8; lanes 13–16, clone SV9). RNA was extracted from normal cells (lanes 1, 3, 5, 7, 9, 11, 13, 15) or MMSV-transformed cells (lanes 2, 4, 6, 8, 12, 14) and twofold serial dilutions of the RNA were spotted and hybridized as described in *b*. Lanes 1 and 2 were hybridized with a *gpt* DNA probe; lanes 5, 6, 9, 10, 13 and 14 were hybridized with a *neo* DNA probe; lanes 3, 4, 7, 8, 11, 12, 15 and 16 were hybridized with a β-actin DNA probe.

Methods. Transformation by MMSV: Cells were seeded in two T-25 flasks (3×10^5 cells per flask); to one of the flasks 5 ml of medium, incubated for 3 h with established MMSV-transformed NIH 3T3 cells, were added. Both normal and infected cells were carried for 1–2 weeks before they were assayed. Transformation by v-*mos*; clones of cells stably transfected with pAZ1005 and expressing the *neo* gene were isolated as described in Fig. 2 legend with the exception that *neo* selection medium was used (600 μg ml^{-1} G418). G418-resistant clones were re-transfected by plasmid pHT25, carrying the v-*mos* gene[24] (gift of G. Van de Woude). The transformed colonies were selected by focus formation and recloned in soft agar.

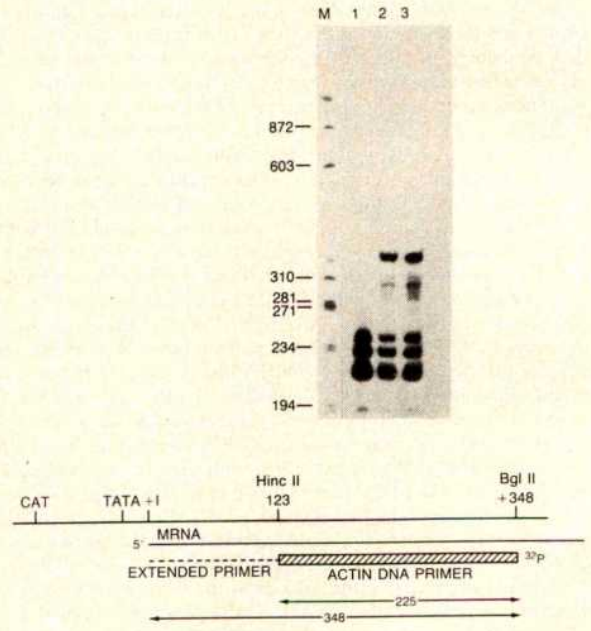


Fig. 4 Levels of chick β-actin RNA in normal and MMSV-transformed NIH 3T3 cells stably transfected by the chicken β-actin gene (pβ6 actin)[19]. A genomic DNA (18 μg) fragment containing the entire chicken β-actin gene cloned in PUC-8 (a gift of B. Paterson) was co-transfected with 2 μg DNA of pSV2-neo[13] into NIH 3T3 cells. G418-resistant clones (~100 colonies) were pooled and transformed by MMSV as described in Fig. 3 legend. Total RNA was extracted by the guanidine hydrochloride method[21] and the levels of chick β-actin RNA were measured using a primer extension method. A single-strand 225-nucleotide *Bgl*II/*Hin*cII fragment of the chicken β-actin cDNA clone, labelled with ^{33}P at its *Bgl*II 5′ end, was used as primer and hybridized to total RNA of untransfected NIH 3T3 cells (lane 1), to untransformed NIH 3T3 cells stably transfected with pAZ1037 (lane 2) and to the same cell transformed with MMSV (lane 3). The primer was extended with reverse transcriptase as described for Fig. 2 and the products were analysed by electrophoresis on a 7 M urea/5% polyacrylamide gel. The bands on the gel were quantitated by microdensitometry; the ratio of the 348-nucleotide cDNA to the 225-nucleotide primer was 0.55 in lane 2 and 0.62 in lane 3.

Table 1 Levels of activity of transfected α2(I) collagen, β-actin and SV40 early promoter in normal and MMSV-transformed cells

	Cell lines	Promoter	CAT specific activity (c.p.m. per mg protein $\times 10^4$) Normal (N)	Transformed (T)	Ratio (N/T)
a	1, AT3-14	Chick α2 type I collagen	19.5	2.8	7.0
	2, AT1		32.0	2.2	14.5
	3, AT2		18.7	1.3	14.4
b	1, AZC1	Mouse α2 type I collagen	25.8	5.4	4.8
	2, AZC2		236.9	60.7	3.9
	3, AZC4		24.1	3.9	6.2
	4, AZC5		86.1	3.9	22.1
	5, AZC6		641.5	504.7	1.3
c	1, AZA1	Chick β-actin	522.1	362.7	1.4
	2, AZA2		118.5	70.6	1.6
	3, AZA3		3.3	2.7	1.2
	4, AZA4		35.4	11.7	3.0
	5, AZA6		41.0	20.3	2.0
d	1, SV1	SV40 early region	214.2	34.7	6.2
	2, SV5		139.3	10.8	12.9
	3, SV6		0.5	0.2	2.5

NIH 3T3 cells were transfected with 10 μg pHO1000 DNA (as described in Fig. 1 legend) (*a*, 1-3) or with 10 μg DNA of pSV2-neo and pAZ1009 (*b*, 1-5), pSV2-neo and pAZ1037 (*c*, 1-5) or pSV2-neo and pSV2-cat (*d*, 1-3). The ratio of pSV2-neo DNA to the other DNA used in co-transfections was 1:10. Individual colonies resistant to mycophenolic acid (*a*) or to G418 (*b*, *c*, *d*) were isolated. Levels of CAT were measured before and after MMSV transformation of these cells as described in Fig. 2 legend.

MMSV. Table 1*d* compares the levels of CAT in these cells before and after MMSV transformation and shows clearly that the enzyme levels are strongly reduced on transformation. In a similar experiment, NIH 3T3 cells were transfected with pSV2-neo alone, and G418-resistant colonies were isolated and transformed by MMSV. Comparison of the levels of *neo* RNA in these cells before and after transformation by MMSV showed that in almost all cell lines the levels of *neo* RNA were decreased after transformation (Fig. 3*d*, lanes 5, 6; 9, 10; 13, 14). These experiments suggest that the activity of the early promoter of SV40 is also inhibited in MMSV-transformed cells and that this effect is not mediated in *cis* via the α2(I) collagen promoter. Because a wide range of changes occurs in the pattern of proteins synthesized in transformed cells, it is not unexpected that another promoter would also be inhibited in transformed fibroblasts. We speculate that the SV40 early promoter contains sequence elements that might interact with one or more proteins which also recognize the α2(I) collagen promoter in fibroblasts. One possible hypothesis is that one or more of these proteins may either be modified or may cease to be synthesized after MMSV transformation.

The decrease in *cat* or *neo* RNA levels in cells where the corresponding genes are linked to the α2(I) collagen promoter is a result of the presence in these cells of an active transforming protein. Indeed, when AT3-14 cells, a cell line in which the α2(I) collagen promoter is fused to the *cat* gene, were transformed with a mutant of the Kirsten murine sarcoma virus, temperature-sensitive for transformation, the levels of CAT activity were decreased at the permissive temperature but not at the non-permissive temperature. These changes parallel the changes in the levels of endogenous α2(I) collagen RNA (data not shown).

Thus, the activity of the α2(I) collagen promoter introduced into NIH 3T3 cells by DNA transfection is strongly inhibited after malignant transformation by the v-*mos* oncogene. This inhibition parallels the reduction in the expression of both the endogenous type (I) collagen and fibronectin[16] genes in fibroblasts. A similar decrease is seen in the activity of the transfected SV40 early promoter, but not in the activity of a transfected β-actin promoter.

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1. Unkeless, J. C. *et al. J. exp. Med.* **137**, 85-112 (1978).
2. Dulak, N. C. & Temin, H. M. *J. cell. Physiol.* **81**, 153-160 (1973).
3. Gottesman, M. M. & Sobel, M. E. *Cell* **19**, 449-455 (1980).
4. Green, H., Todaro, G. J. & Goldberg, B. *Nature* **209**, 916-917 (1966).
5. Levinson, W., Bhatnagar, R. S. & Liu, T. *J. natn. Cancer Inst.* **55**, 807-810 (1975).
6. Hata, R. & Peterkofsky, B. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2933-2937 (1977).
7. Howard, B. H., Adams, S. L., Sobel, M. E., Pastan, I. & de Crombrugghe, B. *J. biol. Chem.* **253**, 5869-5974 (1978).
8. Sobel, M. E., Yamamoto, T., de Crombrugghe, B. & Pastan, I. *Biochemistry* **20**, 2678-2684 (1981).
9. Avvedimento, E. *et al Nucleic Acids Res.* **9**, 1123-1131 (1981).
10. Sandmeyer, S., Gallis, B. & Bornstein, P. *J. biol. Chem.* **256**, 5022-5028 (1981).
11. Ohkubo, H. *et al Proc. natn. Acad. Sci. U.S.A.* **77**, 7059-7063 (1980).
12. Gorman, C. M., Moffat, L. & Howard, B. H. *Molec. cell. Biol.* **2**, 1044-1051 (1982).
13. Southern, P. J. & Berg, P. *J. molec. appl. Genet.* **1**, 327-341 (1982).
14. Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27**, 299-308 (1981).
15. Wasylyk, B., Wasylyk, C., Augeruau, P. & Chambon, P. *Cell* **32**, 503-514 (1983).
16. Vogeli, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 5334-5338 (1981).
17. Mulligan, R. & Berg, P. *Science* **209**, 1422-1427 (1980).
18. Schmidt, A., Yamada, Y. & De Crombrugghe, B. *J. biol. Chem.* **259**, 7411-7415 (1984).
19. Seiler-Tuyns, A., Eldridge, J. D. & Paterson, B. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2980-2984 (1984).
20. Goldfarb, D. S., Rodriguez, R. L. & Doi, R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5886-5890 (1982).
21. Sobel, M. E. *et al Proc. natn. Acad. Sci. U.S.A.* **75**, 5846-5850 (1978).
22. Goldberg, D. A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5794-5798 (1980).
23. Cleveland, D. W. *et al. Cell* **20**, 95-105 (1980).
24. Blair, D. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3504-3508 (1980).

High-affinity binding site for a specific nuclear protein in the human IgM gene

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Proteins binding to specific regions of DNA with high affinity frequently govern or regulate reactions at the gene level[1-8]. We have identified a high-affinity binding site in the immunoglobulin μ gene that binds a specific nuclear protein, and have now characterized it fully using nuclear factor 1 (NF-1), a protein purified from the nuclei of HeLa cells[9,10] and required for the *in vitro* replication of adenovirus (Ad) DNA[9-12]. NF-1 protects a 25-base pair (bp) double-stranded segment of DNA which shares a consensus sequence, 5′ TGGA/CNNNNNGCCAA 3′, with similar

binding sites in the Ad-5 terminal repeat[10–12] and the human c-*myc* gene[13]. Although this site differs from the enhancer region[13–16], a biological function is suggested by the fact that it is DNase I hypersensitive in immunoglobulin-producing lymphoblastoid cells. The binding site for the NF-1 protein in the μ gene, by analogy with the site in the Ad-5 terminal repeat, may represent one component of a cellular origin of replication; alternatively, it may be responsible for the activation of the chromatin in this region.

The immunoglobulin heavy-chain genes represent a set of DNA sequences of intense transcriptional activity in immunoglobulin-producing B cells. They also represent a target for a highly specific translocation event that delivers the oncogene c-*myc* into this locus in most instances of the B-cell malignancy, Burkitt's lymphoma[17,18]. Recently, we have identified five potential regulatory sites in the *myc* gene region on the basis of their DNase I hypersensitivity[13]. Two of these sites,

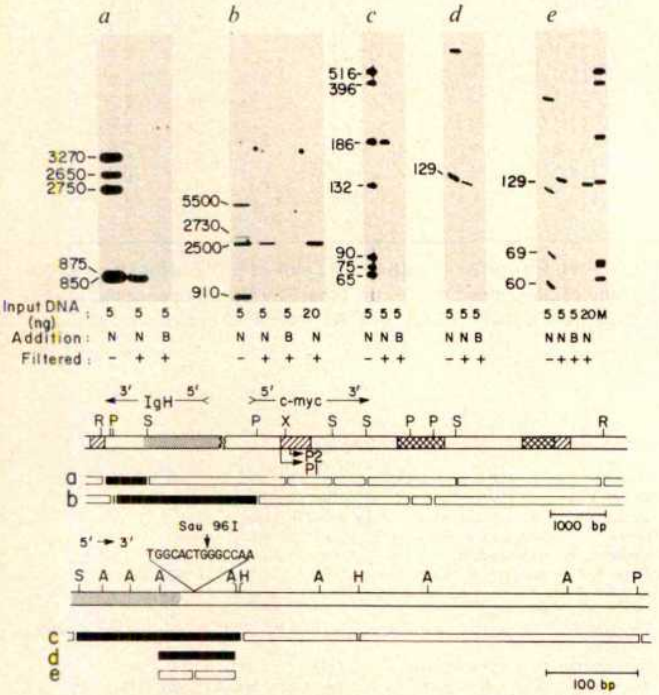


Fig. 1 Mapping of the protein binding sites on DNA fragments overlapping the human IgM and *myc* genes. Recombinant plasmids were cut with restriction enzymes and the resulting fragments were end-labelled using [α^{32}P]-dNTPs (labelled deoxynucleotide triphosphates) and the large fragment of *Escherichia coli* polymerase (Klenow)[13]. Labelled DNA was incubated with a low salt extract from Burkitt's lymphoma (BL22) nuclei (N)[13] for 30 min at 25 °C and passed through nitrocellulose filters. Protein-bound DNA, retained on the filters, was extracted[13] and analysed on agarose gels (*a*, *b*) (+) or sequencing gels (*c*–*e*) (+). The pattern of input DNA was assayed by omitting the nitrocellulose filtration step (−). Background binding of labelled fragments on nitrocellulose filters was analysed by substituting bovine serum albumin (B) for nuclear proteins. To determine protein binding sites, cloned DNA from BL22, overlapping the IgM gene and *myc* gene (pBL22 RI-RI 9.0[16], was cut with *Eco*RI, *Sst*I and *Xho*I (*a*) or with *Pst*I (*b*). *a*, *Eco*RI and *Xho*I sites; *b*, *Pst*I sites were labelled radioactively. For fine mapping, the 600-bp *Sst*I/*Pst*I fragment (sequence is shown in Fig. 2*c*) was cloned into the polylinker of the vector pUC13 (BRL) (pIgHSP0.6) and cut with *Eco*RI and *Hin*fI (*c*). Both types of ends were labelled radioactively. The *Eco*RI site is in the polylinker next to the *Sst*I site. The *Alu*I fragment covering the protein binding site was cloned into the *Sma*I site of M13mp10 RF (M13IgHA80). *d*, *e*, Binding assays where this fragment was cut out with *Eco*RI and *Hin*dIII, the sites which bracket the polylinker. *e*, M13IgHA80 cut with *Eco*RI and *Hin*dIII was recut with *Sau*96I. The 129-bp *Eco*RI/*Hin*dIII fragment is cut into two 60 and 69-bp fragments. The other two fragments are derived from M13mp10 RF. As a positive control for binding, 20 ng M13IgHA80 cut with *Eco*RI and *Hin*dIII were added to the samples to be filtered. Solid bars, DNA fragments bound by nuclear proteins; dotted regions, μ switch region; single-hatched regions indicate non-translated exon sequences. Double hatched regions indicate protein coding sequences. P1 and P2, the two promoters of the *myc* gene. A, *Alu*I; R, *Eco*RI; H, *Hin*fI; P, *Pst*I; S, *Sst*I; X, *Xho*I. As a relative molecular mass marker (M) in *e*, the plasmid pIgHSP0.6 had been digested with *Hin*fI and *Eco*RI. The visible bands indicate the following sizes: 516, 396, 186, 132, 84, 78 and 68 bp.

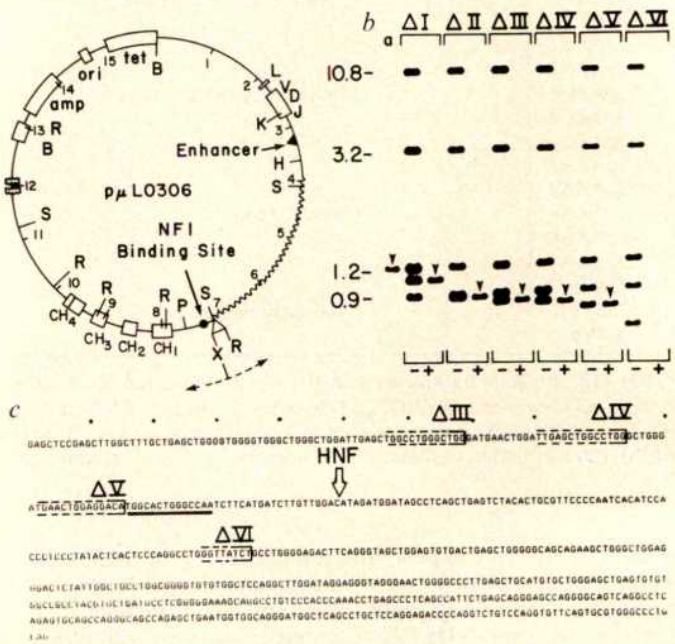


Fig. 2 Fine mapping of the NF-1 binding site in an IgM gene. *a*, Construction of pμL0306: B, *Bam*HI; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sst*I. Wavy line, μ switch region; open boxes drawn with wavy lines, IgM membrane exons, the exact location of which is not known; open boxes with straight lines represent IgM exons. *b*, Mapping the protein binding sites on the IgM plasmids carrying the *Bal*31 deletions; the sizes of the *Eco*RI fragments not subjected to *Bal*31 deletions are indicated in kb. The first lane (*a*) shows the filter-bound *Eco*RI fragment of the starting plasmid pμL0306 (filter-bound fragments are always indicated by an arrow). The numbers above the upper brackets indicate the different plasmids used. Below the lower brackets, lanes with non-filtered (−) and filter-bound (+) DNA are indicated. *c*, DNA sequence of the 603-bp *Sst*I/*Pst*I fragment. The DNA sequence was derived by using the dideoxy chain terminating method according to the BRL Manual (BRL, Inc.). The brackets indicate the endpoints of the various deletions. Sequences to the left of the brackets were deleted and replaced with *lacZ* DNA. The numbers above the brackets indicate the respective plasmid. The underlined sequence is the core of the proposed NF-1 binding site (see Fig. 3*a*). The arrow above the sequence indicates the midpoint of the DNase I hypersensitive region (HNF) (see Fig. 4).

Methods. *a*, A pBR322 molecule was linearized by *Bam*HI and, in a series of steps, ligated to three pieces of DNA from human μ libraries to produce a cloned rearranged human μ plasmid. The region from the *Bam*HI site at ~12.8 kb to the *Hin*dIII site at ~3.7 kb came from a germline library, the region from this *Hin*dIII site to the *Kpn*I site at ~2.7 kb from a lymphoma library (line LR35) and the region from this *Kpn*I site to the *Bam*HI site at 0 kb from a closely related lymphoma library (line LR36). Into the germline piece was inserted (as shown) a 268-bp *Sst*I/*Sst*I fragment containing M13mp11 sequences from the *Sst*I site in the polylinker to the *Pvu*II site in the *lac*I gene (the latter converted to an *Eco*RI site with an *Eco*RI linker), joined to pBR327 sequences from the *Eco*RI site to the *Cla*I site (the latter converted to an *Sst*I site by blunting the ends with Klenow polymerase and adding an *Sst*I linker). The unique *Xho*I site had been produced by cloning an *Xho*I linker into the M13mp11 *Sma*I site. Not all restriction sites are shown for all enzymes. Following *Bal*31 deletion starting at the unique *Xho*I site, the plasmids were re-ligated after blunting the ends with Klenow polymerase. *b*, Six plasmids carrying different deletions were cut with *Eco*RI labelled by fill-in and incubated with nuclear extract. Non-filtered (−) as well as filter-bound (+) DNA was subjected to agarose gel electrophoresis (for details see Fig. 1).

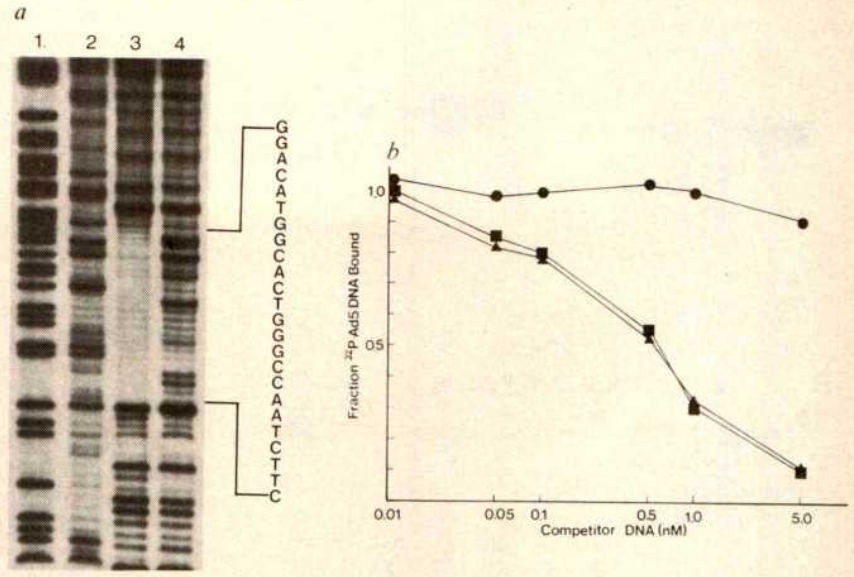


Fig. 3 *a*, DNase I footprint of the NF-1 binding site in the IgM gene. *b*, Competition nitrocellulose filter-binding assays with Ad-5 and IgM NF-1 binding sequences.
Methods. *a*, A ^{32}P-end-labelled DNA fragment from M13IgHA80 RF DNA containing a NF-1 binding site was subjected to limited DNase I digestion in the absence (lane 3) or presence (lane 4) of the phosphocellulose fraction[10] of NF-1 protein and the products were analysed on an 8% polyacrylamide urea sequencing gel. G+A (lane 1) and A+C (lane 2) sequencing reactions of the fragment were run in parallel lanes to determine the sequence of the region protected by the protein. M13IgHA80 RF DNA was linearized by restriction with *Eco*RI and the 3′ recessed ends of the fragments were filled-in and labelled using DNA polymerase I large fragment (Klenow) and [α-^{32}P]dATP (3,000 ci mmol^{-1}). The labelled DNA fragments were cut with *Hin*dIII and the fragment containing the NF-1 binding site isolated by electrophoresis in a 2% agarose gel and extracted by electroelution followed by chromatography through NACs resin (BRL). Protein–DNA binding was carried out at 0 °C for 20 min in a reaction mixture (40 μl) containing 50 mM HEPES (*p*H 7.5), 5 mM MgCl, 1 mM dithiothreitol DTT, 250 μg m^{-1} bovine serum albumin, 125 μg ml^{-1} *E. coli* DNA, 200 mM NaCl, 15 μl phosphocellulose I fraction of NF-1 and 1 ng ^{32}P-DNA fragment. The reaction mixtures were incubated with DNase I (0.075 μg) for 1 min at 23 °C, extracted with neutralized phenol and, after ethanol precipitation, the reaction products analysed as described above. *b*, The binding affinities of NF-1 to Ad-5 and IgM DNA were compared by competition nitrocellulose filter-binding assays as described previously[10]. Purified NF-1 (200 ng) and 5 fmol *Eco*RI-restricted ^{32}P-labelled pMDC10 DNA (contains 365 bp of Ad-5 left-terminal sequence) were incubated with varying amounts of unlabelled form I pMDC10 and PIgHSP0.6. The concentration of labelled pMDC10 *Eco*RI bound to NF-1 was determined by nitrocellulose filter binding. The fraction bound was calculated as the ratio of Ad-5 DNA bound to the filter in the presence and absence of competitor DNA. The following DNAs were used as competitors: ●, pUC9 (plasmid DNA); ■, pMDC10 (Ad-5/NF-1 binding site); ▲, pIgHSP0.6 (IgM/NF-1 binding site).

a putative repressor binding site and a weak NF-1 binding site, are not associated with the translocated and deregulated *myc* gene in the Burkitt's lymphoma cell line, BL22 (refs 19, 20). Therefore *myc* activation could be the result of *cis*-acting sequences in the immunoglobulin heavy-chain locus (*Igh*). To identify such DNA sequences we sought to find regions in the translocated immunoglobulin μ segment which might interact specifically with nuclear proteins. Using a nitrocellulose filter binding assay[13,21,22] and cloned DNA (pBL22 RI-RI 9.0; see Fig. 1), including the IgM/*myc* crossover region in BL22 (ref. 16). We discovered a fragment of DNA between the μ switch and the μ constant region binding specifically to protein(s) derived from a low-salt BL22 nuclear extract[13]. The binding region is in a 603-bp DNA segment between a *Pst*I and *Sst*I site (see Fig. 1*a*, *b*). Subcloned restriction fragments of this 603-bp fragment were assayed and the binding site further mapped to a 80-bp *Alu*I fragment (Fig. 1*c*, *d*). Recutting a 129-bp fragment containing the 80-bp *Alu*I fragment with *Sau*96I destroyed the ability of the resulting fragments (69 and 60 bp, see Fig. 1*e*) to be retained in the filter binding assay, suggesting that the binding site either contains the *Sau*96I site or lies very close to it. The binding site is also present in the germline configuration of the *Igh* locus (Fig. 2), indicating that mutational events in BL22 are not responsible for its appearance. Further information about the location and nature of the protein binding site was obtained using *Bal*31 deletions, sequence analysis and DNase I footprinting *in vitro* (Figs 2, 3). Using a normal μ gene, we constructed deletions starting from a unique *Xho*I site cloned into the *Sst*I site at the 3′ end of the μ switch (see Fig. 2*a*) and extending up to and through the putative binding site (Fig. 2). Whereas deletions ΔI–ΔV do not destroy the binding site, deletion ΔVI, which ends 3′ of the normally bound *Alu*I fragment (Fig. 1*d*), abolishes binding (Fig. 2*b*).

The sequence of this region suggested that the bound nuclear protein might be NF-1. This protein binds to two homologous sequences in Ad-5 (refs 10,13) and the c-*myc* gene[13]. Here we show that the homology extends to a sequence in the μ-gene fragment which is recognized in the filter binding assay (Figs 1*d*, 2*c*). To confirm this, the pure NF-1–DNA interaction was visualized by DNase I footprinting analysis (Fig. 3*a*). Although NF-I protects ~25 bp, sequence comparisons suggest that only a core sequence in the centre of the protected area is necessary for the specific protein DNA interaction (Fig. 2). By comparing the sequence in the IgM gene protected by NF-1 (Fig. 3*a*) with the published NF-1 sites in Ad-5 (refs 10,12) and the *myc* gene[13] and unpublished sites in other adenovirus serotypes as well as in the human polyomavirus JC and the human and simian cytomegalovirus (D.R., P.R., G. Hayward, P. Padmanablan and L.H., unpublished results), a consensus sequence can be derived (5′ . . . TGGA/ CNNNNGCCAA . . . 3′). In addition, replacement of the sequences upstream of the TGG . . . (Fig. 2*c*) with *lacZ* sequences (data not shown) in deletion mutant ΔV does not interfere with binding of NF-1 (Fig. 2*b*). A DNA binding protein from chicken oviduct nuclei recognizes a similar sequence in the chicken lysozyme gene region[23].

The binding site exhibits a 2-fold axis of symmetry exemplified by the TGGC . . . GCCA sequences. High binding affinity occurs when this symmetry is most pronounced, as in the IgM and Ad-5 binding sites[10,13]. Using an equilibrium competition assay, we show that the IgM and Ad-5 binding site affinities are nearly equal (Fig. 3*b*) and an order of magnitude stronger than the c-*myc* binding site, which exhibits only an approximate symmetry[13]. The 2-fold symmetry axis in the binding site suggests that NF-1 binds as a dimeric form mechanistically similar to repressor protein DNA interactions[24]. Although it is not known whether NF-1 purified from HeLa cells and the binding protein(s) from B cells are identical, we have shown that both binding activities recognize the same synthetic binding site cloned into a plasmid vector (L.H. and L. Davis, in preparation).

To assign biological significance to the NF-1 binding site in the IgM gene, we provide evidence that NF-1 also binds to this element *in vivo*. The localization of DNase I hypersensitive sites in chromatin can identify potential protein binding sites[3,25,26]. Therefore, we mapped the DNase I hypersensitive sites in the IgM gene in lymphoblastoid cells and in BL22 (Fig. 4). A probe from the μ constant region detects two DNase I hypersensitive sites in the lymphoblastoid cell line 8392 (Fig. 4*A*, *a*). One in the μ switch, approximately where the *myc*μ translocation point in BL22 is found, and one near the NF-1 binding site. NF-1 binds ~3.1 kilobases (kb) 5′ to the *Xba*I site (Fig. 4*B*, *a*) and

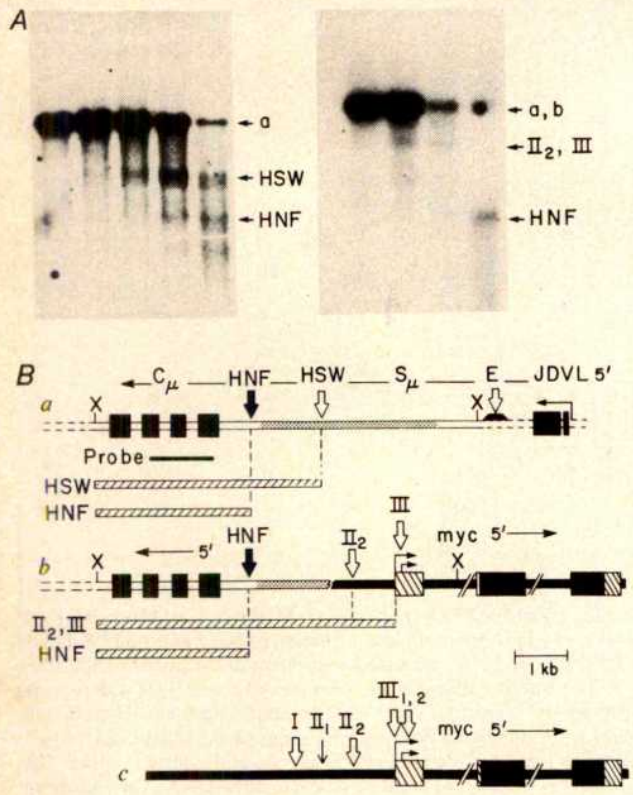


Fig. 4 *A*, Mapping of DNase I hypersensitive sites near the c-*myc* gene and IgM gene in the lymphoblastoid cell line 8392 (left) and the Burkitt's lymphoma cell line BL22 (right). Nuclei of both cells were digested with increasing amounts of DNase I as described previously[13] and then 15 μg of isolated genomic DNA restricted with *Xba*I, electrophoresed on a 0.8% agarose gel, blotted onto nitrocellulose and hybridized. A 1.2-kb *Eco*RI fragment overlapping the μ constant region was used as hybridization probe. *B*, The probe hybridizes to a germline IgM fragment (*a*) and to a fragment of similar size from the IgM *myc* translocation product (*b*). The DNase I hypersensitive sites are labelled HSW (hypersensitive switch), HNF (hypersensitive NF-1 binding site), E (enhancer)[13-16], I, II1, II2, III1, and III2 as described previously[13] and their positions are indicated on the map (*X*, *Xba*I). *a*, *b*, Solid arrow indicates the binding site for NF-1; cross-hatched bars, sub-bands in the cell lines 8392 (*a*) and BL22 (*b*). Protein coding regions of both the *myc* gene and the IgM gene are indicated by solid boxes, nontranslated regions of exons by cross-hatched boxes. Dotted regions correspond to the μ switch. The *myc* gene is not drawn to scale. *c*, Pattern of DNase I hypersensitive sites in the germline *myc* is shown[13].

therefore falls into the DNase I hypersensitive region represented by a sub-band 3,050–3,200-bp long (Fig. 4*B*, *a*). The same probe picks up two sub-bands in BL22 (Fig. 4*B*, *b*), one representing the DNase I hypersensitive region at the NF-1 binding site and one representing closely linked sites in the *myc* gene translocated into the IgM region. This band has a size of ~5 kb and presumably corresponds to the sites II2, III1 and III2 (Fig. 4*B*, *b*, *c*) occurring in the 5′portion of the *myc* gene[13]. The co-localization of DNase I hypersensitive sites and NF-1 binding sites in the *myc*[13] and IgM genes suggests a possible NF-1 DNA interaction *in vivo*.

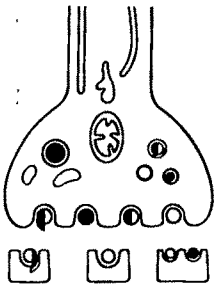
The function of the μ NF-1 binding site remains to be defined. It could be similar to that of the analogous sequence in adenovirus, that is, acting as one component of an origin of replication. In the HeLa cell genome, the frequency of high-affinity NF-1 binding sites is one per 100 kb[27], a spacing similar to that of cellular origins of replication[28]. Alternatively, these sites could be involved in transcriptional activation, although this is not consistent with the distribution of NF-1 binding sites in the immunoglobulin heavy-chain locus. The NF1 site is in the first intron of the μ gene just 3′ to the μ-switch region. A search in clones overlapping the γ and ε switch and constant regions failed to reveal any binding sites (data not shown). During B-cell development, a series of recombination events leads to a switch in heavy-chain constant-region class from μ/δ to either γ, ε or α[29,30]. After switching from μ/δ to any other constant region, the μ NF-1 binding site would be deleted from the resulting active heavy-chain gene. By contrast, the canonical *Igh* enhancer 5′ to the μ switch is retained during heavy-chain class switching[14-16]. This, together with the fact that equivalent binding sites do not occur at corresponding positions in the γ and ε genes, makes it less probable that NF-1 acts as a simple transcriptional enhancer. On the other hand, the loss of the μ NF-1 binding site during heavy-chain class switching could indicate that, in the *Igh* region, NF-1 is a necessary component for this 'dead end' recombination process. We are left with another, equally attractive, possibility, that the NF-1 binding sites have a more universal role in the activation of chromatin during early stages of cell differentiation, suggested by the observation that the NF-1 binding sites in the human *myc*[13] and IgM genes are located in DNase I hypersensitive regions, areas associated with the active state of chromatin.

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1. Gilbert, W., Maizels, N. & Maxam, A. *Cold Spring Harb. Symp. quant. Biol.* **38**, 845–855 (1974).
2. Maniatis, T. *et al. Cell* **5**, 109–113 (1975).
3. Dynan, W. S. & Tjian, R. *Cell* **35**, 79–87 (1983).
4. Jones, K. A. & Tjian, R. *Cell* **36**, 155–162 (1984).
5. Compton, J. G., Schrader, W. T. & O'Malley, B. W. *Proc. nat. Acad. Sci. U.S.A.* **80**, 16–20 (1983).
6. Karin, M. *et al. Nature* **308**, 513–519 (1984).
7. Renkawitz, R., Schutz, G., von der Ahe, D. & Beato, M. *Cell* **37**, 503–510 (1984).
8. Chandler, V. L., Maler, B. A. & Yamamoto, K. R. *Cell* **33**, 489–499 (1983).
9. Nagata, K., Guggenheimer, R. A., Enomoto, T., Lichy, J. H. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6438–6442 (1982).
10. Rawlins, D. R., Rosenfeld, P. J., Wides, R. J., Challberg, M. D. & Kelly, T. J. Jr *Cell* **37**, 309–319 (1984).
11. Nagata, K., Guggenheimer, R. A. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6177–6181 (1983).
12. Guggenheimer, R. A., Stillman, B. W., Nagata, K., Tamanoi, F. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3069–3073 (1984).
13. Siebenlist, U., Hennighausen, L., Battey, J. & Leder, P. *Cell* **37**, 381–391 (1984).
14. Hayday, A. *et al. Nature* **307**, 334–340 (1984).
15. Rabbitts, T. H., Hamlyn, P. H. & Baer, R. *Nature* **306**, 760–765 (1983).
16. Bannerji, J., Olson, L. & Schaffner, W. *Cell* **33**, 729–740 (1983).
17. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7837–7841 (1982).
18. Leder, P. *et al. Science* **222**, 765–771 (1983).
19. Battey, J. *et al. Cell* **34**, 779–789 (1983).
20. Taub, R. *et al. Cell* **36**, 339–348 (1984).
21. Riggs, A. D., Bourgeois, S. & Cohn, M. *J. molec. Biol.* **53**, 401–417 (1970).
22. Jack, R. S., Gehring, W. J. & Brack, C. *Cell* **24**, 321–333 (1981).
23. Borgmeyer, U., Nowock, J. & Sippel, A. E. *Nucleic Acids Res.* **12**, 4295–4311 (1984).
24. Pabo, C. & Lewis, A. *Nature* **298**, 443–447 (1982).
25. Shermoen, A. W. & Beckendorf, S. K. *Cell* **29**, 601–607 (1982).
26. Emerson, B. M. & Felsenfeld, G. *Proc. natn. Acad. Sci. U.S.A.* **81**, 95–99 (1984).
27. Gronostajski, R. M., Nagata, K. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4013–4017 (1984).
28. Lewin, B. M. *Gene Expression 2* 2nd edn (Wiley, New York, 1980).
29. Leder, P. *Sci. Am.* **246**(5), 102–115 (1982).
30. Shimizu, A. & Honjo, T. *Cell* **36**, 801–803 (1984).

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DNA Methylation

Biochemistry and Biological Significance

Editors: **A. Razin, H. Cedar, A. D. Riggs**
1984. 73 figures. XIII, 392 pages
(Springer Series in Molecular Biology)
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Eastern and Western approaches

Roy MacLeod

The Touch of Midas: Science, Values and Environment in Islam and the West.
Edited by Ziauddin Sardar.
Manchester University Press/Longwood University Press: 1984. Pp.253. £20, $35.

VISITORS to the Science Museum in London during the spring and summer of 1976 were greeted by an unusual exhibition. Conceived by Professor S.H. Nasr, then Director of the Iranian Academy of Science, there was on view a rich display of science and technology in Islam; a display of artefacts, but also of ideas and ambitions. If science exists with such powerful resonances in traditional Islam, the unstated message read, what could it say to the West today?

For generations, it has been the received wisdom in our schools that the Muslim world had, through the Dark Ages in Europe, even from the end of Alexandria to the rise of the Renaissance, acted as a cultural "carrier" — a custodian of the Graeco-Roman scientific tradition. Western eyes, closed to Islam after the fall of Constantinople in 1453, looked instead to passages and conquests overseas, eventually bringing the fruits of exploration and empire in tribute to a Europe emboldened by the "new philosophy" of the scientific revolution. In our schools we averted our eyes from Islam, portrayed as an ossified metaphysics, unproductive in the age of men committed to "the effecting of all things possible".

Yet, if one enquired, the position was never so simple. Surely the sources of discovery in Islam drew from the same wells as those which revived the "West"? Surely no historian could ignore the fundamental contributions to mathematics and medicine, optics and astronomy, pharmacy and biology, engineering and anatomy, associated with the Islamic world from North Africa to India? No student of Sarton or Miéli could ignore the discoveries of Ibn al-Haitham (Alhazen), Avicenna, al-Bīrūnī, or even Ibn al-Nafis, even if, in our pages, where ideas count for more than men, they hovered like restless shadows between the heat and cold of the desert, concealed by enveloping gardens of mystic lore. Still, Islam, as a philosophical system, had its specialists and as such could be left to one side. Language and mystery silenced our doubts; our ignorance flourished.

Within the past 15 years, several factors have helped to alter this dismal prospect. First, there came the weight of fresh scholarship, notably that of David King, David Pingree and G.S. Kennedy, and the late Willy Hartner, paralleling and complementing Joseph Needham's work on China and A. Rahman's on India. This compelled European and American attention to long-neglected sources, to evidences of scientific achievement associated with what after the seventh century AD became the Muslim world. In the process, the "Middle Ages" acquired a totally new connotation, as did our sense of "science" as a unique feature of Western civilization. As this process was at work among scholars, there came what appeared to be a renaissance of an "Islamic cultural nationalism", a phenomenon particularly noticeable in Pakistan and Iran (and one of which the Science Museum was an indirect beneficiary). That renewed interest, coupled with the oil crisis of the mid-1970s, lifted scholars with no previous experience of the Middle East or Central Asia into asking fresh questions about Islam, its political sects, its Qur'anic precepts and its conceptual and methodological legacy.

The values implicit in the legacy have now become a central subject of great importance to the West. Through the 1970s, with increasing international concern for the conservation of the global environment, came a "crisis in science", in large measure reflecting disillusionment with the politics of Western technology. This in turn bred sustained reflection and recrimination among historians and other scholars. For three centuries, the gift of Western science, the "touch of Midas", had held promise of eliminating poverty and materially developing the world; in the process, perhaps scientific solutions would eventually be found to eliminate even the boundaries and burdens of ideology. Alas, however, as the experience of the world revealed, the eutetic gifts of Midas had serious shortcomings. Western science, with its powerful belief in objective, value-free knowledge, taught us that what can be discovered must be discovered, without teaching us at what cost. Western technology had, as its basis, a categorical imperative — that everything which can be done will be done. But we were unprepared for the compromise in values this sometimes entailed. With our triumphs, now competed perils.

If the "Occident" was thus caught in a dilemma of its own making, what of Islam? Here, the question was not trivially one of cultural chauvinism, or the search for scientific priorities. The Muslim world had felt the force of the West's scientific and industrial revolutions, and now felt the weight of technological dependence. Any clash of values experienced in the West would be multiplied in the world of Islam. Where would lie the way forward, between value systems long at daggers drawn?

It is to this question that Ziauddin Sardar addresses himself in this first major publication of a programme of symposia on "Islam and the West". In conferences during 1981 and 1982, sponsored by UNESCO and IFIAS, and Islam and the West International, Sardar and 13 contributors confronted Islamic and Western approaches to science and explored answers to two questions. First, can in Islam be found a new synthesis, capable of offering Occidental rationality a humane vision of knowledge, to the benefit of all mankind? Second, can there be a distinct "Islamic science" which in some way can help reunify knowledge and responsibility?

If there is a right answer to either question, the key lies in understanding the relation between Islamic principles, precepts and practice. As M. Husain Sadr and others explain, Islam is a total epistemological system; there is no dichotomy between philosophy and science, economics and politics, or religion and society. As a holistic system, it permeates all human activity. There is, therefore, no distinction between the ends and means of knowing, and between knowing and applying that knowledge. Islamic teaching incorporates a framework of concepts — including the unity of God, trusteeship, worship, civilization, social justice and public interest. These concepts, if properly acted upon, will surely promote both social and ethical accountability. In pursuit of mutual understanding, participants explored Western and Islamic values and perspectives, debated interpretations of history, and grappled with the task of creating a common agenda.

There can be no doubt of the interest generated by their discussion, or of its importance in bridging discourses long distinct. What emerged is a shared view that ethical insights can and must be sought from Islam. But there are difficulties, as all participants recognized. For a start, as J.R. Ravetz asked, can we be certain about the problem? What, precisely, is the "crisis of science"? Is it a singularly Western phenomenon? If so, surely the ethical dilemmas of Western science must be faced within the Western tradition? Is science, as knowledge, in itself good, requiring only a renewed piety to qualify abuses and bring it to humane fulfilment? Or is science fundamentally a secular activity, owing allegiance to its own self-regulating criteria, its paymasters, and the interests and values of its practitioners? Further, what precisely is the range of these "values"? Is, as Munawar Anees would argue, God the source of all that is "valuable"? If so, in what sense are human values subjective? The bewildering complexity of such theological conundra threatens even the willing traveller.

For some, it may be that to travel hopefully is more important than to arrive. But a destination of a kind comes into view in the applications of the new "Islamic im-

perative" to the built environment. Essays by Lloyd Timberlake, Alison Ravetz, S. Parvez Munzoor and S. Gulzar Haider help both to articulate the environmental ethics of Islam, and give them practical expression. Haider's model of "habitat and values" traces architectural designs in houses and cities that embody precisely those principles of integrity, purpose and clarity which have for centuries fascinated Western travellers to the Middle East. Embracing metaphors of time and structure, he offers a bold synthesis in sand and stone.

Unavoidably, as Robert Walgate summarized, ethical exhortation can appear exceedingly nebulous when one is confronted by practical issues — from militant Islamic nationalism, to the eager acquisition of Western technology apparently regardless of what would be in the West understood as "public interest". What *can* Islam offer Western science? The jury is still undecided. The ethical debates of Western science during the past two decades have not penetrated Islam. Under the circumstances, what are the implications, ethical or economic, of the "Islamization" of science and technology, as espoused here by Ali Kettani? The faith has different aspects, revealing themselves sometimes as fundamentalism, sometimes as mysticism and sometimes worldly-wise pragmatism. Which is to form our new view of science in Islam? Presumably, Islamic science must, in a practical sense, "work". We seek operational knowledge. How do we obtain it? Can there really be no sociobiology in Islam? And what of technology — can there be an Islamic bulldozer, and, if so, how would its construction or function differ from Massey-Ferguson's finest? Finally, what are the political dimensions of Islamic science? As Robert Walgate reflected, the cult of the expert gives great power in Islam to the *Ulamā*, just as the cult of expertise is given great power (and accountability) in the West. If one wants gold, can one escape the fate of Midas?

Mr Sardar would not claim these questions have been fully answered. The discussion must go on, East and West, aided by such promising sponsorship. From it arises a challenge for Muslim scholars to find ways of encouraging the translation of beneficent principles into practical action, whether in fashioning the human environment, directing the goals of medical research, or in the conduct of everyday life. As such, the task is clearly not confined to Islam. □

Roy MacLeod is Professor of History at the University of Sydney.

Biology of paradise

Mark Williamson

Ecology and Biogeography in Sri Lanka.
Edited by C.H. Fernando.
Dr W. Junk: 1984. Pp.505. Dfl. 265, $110, £67.50.

Biogeography and Ecology of the Seychelles Islands.
Edited by D.R. Stoddart.
Dr W. Junk: 1984. Pp.691. Dfl. 300, $115, £76.50.

BOTH Sri Lanka and the Seychelles have claims to be paradise on Earth. Both also have much to offer to biogeographers and ecologists, are tropical and are in the Indian Ocean. But while Sri Lanka is a large continental island (65,000 km^2) with high mountains (up to 2,500 m), some rainforest and a long history of cultivation, the Seychelles are minute (a total land area of only some 400 km^2), in the middle of the ocean and include low coral islands, "high" limestone islands up to 8 m (of which Aldabra is the best known) and the uniquely isolated high granitic islands, rising to 900 m.

These two volumes, the latest in the *Monographiae Biologicae* series, continue a tradition of discussing interesting islands. Past volumes have covered the Canaries, New Guinea, Newfoundland and the Pityusics (Balearics). However, the intent of the series is not clear to me. The monographs are advertised as "hydrobiology", and most deal with rivers and lakes, but a case study of an ancient man-made lake in Sri Lanka (there are no natural lakes) comes in the *Developments in Hydrobiology* series from the same publisher.

So what features of island life would one expect to be covered in such a monograph? Something on geology, certainly, and something on climates. Islands have interesting relic and endemic species, so there should be something on their origin and, by now, on the rate of evolution. Since Darwin, biologists have been interested in dispersal to islands, a topic often contrasted nowadays with vicariance biogeography. The number of species, their population sizes, their turnover by immigration and extinction, the representation of this in species-area curves, and by incidence and prevalence curves, are all topics of current interest. Questions of adaptation and life history strategy naturally arise from all this, and indeed islands are generally regarded as useful experiments. But not as yet, it would seem, in the Indian Ocean.

This is not to say that some of these topics are not dealt with, and indeed dealt with quite well, in the Seychelles volume, and some of them are touched upon in the volume on Sri Lanka. But the difficulties seem to be two. The first — and this applies particularly to Sri Lanka — is that taxonomy is still fairly primitive, even in groups like mammals. But the major problem is the complexity of geological history in the Indian Ocean. During the Tertiary it resembled a dodgem stand at a fair — land whizzing in all directions, though with a pattern discernible overall; collisions here, pieces stuck there. As Braithwaite says, "if displacements in the Atlantic had been so elaborate the verification of concepts of sea-floor spreading might have been delayed for years". While the authors in the Seychelles volume do their best to get to grips with the geological background, those in the Sri Lankan volume seem mostly unaware of these phenomena, except for the occasional mention of Gondwanaland.

The volume on Sri Lanka is not so much a monograph, more a scrapbook. Interesting topics crop up, but there is little coherence. The worst chapter is "Freshwater Invertebrates, Some Comments", which comprises just two and a half pages of references to previous works. There is an account of grasslands, but not of rainforest. There are chapters on the ecology of rice fields, on Monogenea from freshwater fishes, on coastal lagoons, on parasites of the endemic and relic vertebrates, but the only vertebrate group dealt with is mammals. There is emphasis on freshwater habitats and organisms, and also on the human aspects, such as malaria, man-made lakes and land use. It is difficult to steer a course between a superficial account and a tedious catalogue, but this volume too often hits the rocks on both sides.

In contrast, the volume on Seychelles is comprehensive and stimulating, though one weakness is that the information on, say, the areas of different islands is scattered through various chapters. There are accounts of the geology and climate, coral reefs and the marine fauna, vegetation and floristics, land molluscs, terrestrial arthropods, herpetofauna, birds and on man's influence. The high Seychelles are the only small granitic islands in the middle of an ocean, and they have a remarkable fauna and flora to suit. The absence of amphibians comes into the standard definition of an oceanic island, but on the Seychelles there are five species of frogs and seven species of caecilians, burrowing legless forms; the latter at least Gondwanan relics. An introductory historical chapter includes good maps, a table of diversity and endemicity, and species-area curves.

It is really rather odd to find two volumes in the same series, appearing at the same time, which differ so much in quality. For while the monograph on Sri Lanka will be of short-lived interest, even to specialists, that on the Seychelles may well be a standard reference work for many years. □

Mark Williamson is Professor of Biology at the University of York.

● Also published by Dr W. Junk in the series *Monographiae Biologicae*, volume 56, is *The Amazon: Limnology and Landscape Ecology of a Mighty Tropical River and its Basin*, edited by Harald Sioli. The series editor is H.J. Dumont and the price is Dfl. 450, $172, £114.50.

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High Performance Liquid Chromatography in Biochemistry

edited by **A. Henschen, K.-P. Hupe, F. Lottspeich** and **W. Voelter**

1985. Approximately XII, 564 pages. Hardcover. DM 198.–/US $ 88.00. ISBN 3-527-26057-9

It is hard to imagine what it would mean to do research in chemistry if chromatography were not available as an analytical tool. In particular, liquid chromatography in all its modes has become indispensable. Its high performance version, known as HPLC, has brought new dimensions to analytical work. Separation efficiency, speed of analysis and level of detection have shown dramatic improvements. The method can now be performed on a microscale.
This book gives an introduction to the theoretical and instrumental principles of HPLC and a detailed treatment of its application to compounds of biochemical interest. The first chapters deal with fundamental chromatographic relationships, the column and the instrumentation used in HPLC. Among the compound classes considered in the following chapters are amino acids, peptides, lipids, carbohydrates, biogenic amines, porphyrins, steroids, vitamins and organic acids.
Two lists of symbols and abbreviations facilitate the use of the literature in the field, and the detailed subject index guarantees fast and reliable access to the wealth of information provided by the book.

The Practice of Quantitative Gel Electrophoresis

by **A. Chrambach**

Series: **Advanced Methods in the Biological Sciences, Volume 1**

1985. Approximately XVI, 184 pages, 52 figures and 21 tables.
Hardcover. Approximately DM 110.–/US $ 43.60. ISBN 3-527-26039-9

Electrophoresis is one of the most important methods for the investigation of biological materials and probably the most efficient procedure for the separation and detection of proteins and other charged species. Special methods which can be summarized as gel electrophoresis, include polyacrylamide and agarose electrophoresis, isotachophoresis and electrofocusing.
This book presents gel electrophoretic procedures which are generally applicable to the identification and isolation of charged molecules. All methods have proved reliable and are used in daily laboratory work. Also, the equipment required for gel electrophoresis is described in detail. For items that are not commercially available, the reader gets all the information needed to do the construction himself. Theoretical aspects are covered to the extent that they are indispensible for the application of a method. A comprehensive index completes the book and makes it a reliable reference work.

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Around the world in eighty years

Harriet Guest

Voyage into Substance: Art, Science, Nature and the Illustrated Travel Account, 1760-1840.
By Barbara Maria Stafford.
MIT Press: 1984. Pp.645. $39.95, £41.75.

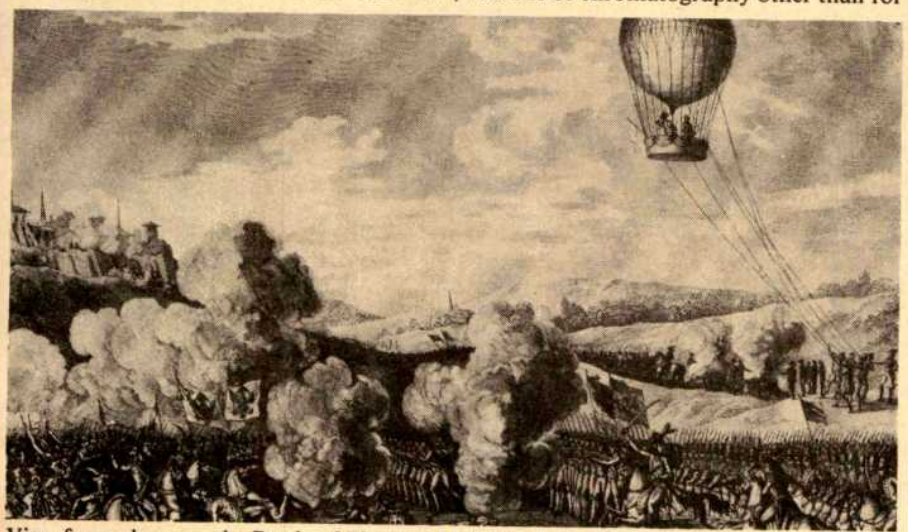
FROM its foundation in 1660 the Royal Society was aware of the capital value of the "*Philosophical* stock" accumulated by "Voyages into all parts of the World". Following the invention of a reliable marine chronometer, the later eighteenth century saw a proliferation of voyages (and land expeditions) from Europe, one of whose aims was the garnering of further philosophical stores. This aspect of exploration and travel accounts as investigations and surveys of nature forms the subject of Professor Stafford's *Voyage into Substance*.

The book is, as its title suggests, a journey into a considerable range of subject matter: Professor Stafford discusses and refers to a mass of accounts, and an almost overwhelming variety of locations, ranging from the Sahara to the Arctic, from the microscopic to the planetary, from the mine shaft to the balloon. Almost inevitably, however, this vast range poses problems of organization and method. The author argues that the diverse representations of nature that fall within the scope of her study are unified as expressions of the "scientific gaze". This gaze is achieved through a willed act of seeing, which somehow voluntarily denudes itself of cultural content or accretions, as "scientific scrutiny recaptured the innocent eye of the archetypal encounter with the earth". She claims that "scientific perspicacity... inaugurated a new visual habit of plainness or real transparency", and disclosed a nature which declared its own history and meaning. This innocent vision finds its expression in "a verbal (and pictorial) scientific style", of "masculine" plainness and accurate particularity, where "Art as scientific language, as limpid and democratic medium for the transcription of ipseity, does not self-expressively alter the pure presence of the properties it remands". It was evidently a style very different from Professor Stafford's.

The extent to which the procedures, at least, of the scientific enterprise were involved in and informed by its cultural contexts may be obscured by Professor Stafford's very selective notion of natural philosophy. The Royal Society, in its *Secret Instructions* to Cook, enjoined him to observe and collect a considerable variety of inanimate natural phenomena, but it also instructed him to "observe the Genius, Temper, Disposition and Number" of the native peoples he encountered. A large proportion of the 270 illustrations to this book carry out the same instruction: when, for example, William Hamilton illustrated the eruption of Vesuvius, he included in his aquatints some Neapolitan spectators of the event, and their expressive figures clearly invite us to see it as more than an object of scientific scrutiny. But Professor Stafford comments only on the "probing scrutiny" of the artist's gaze, and indeed almost never refers to the interpretative functions of human figures and compositional structures in landscapes. She quotes Georg Forster's intention "to throw more light upon the nature of the human mind, and to lift the soul into that exalted station, from whence the extensive view must justify the ways of God to man...", and its triple literary allusion to Pope, Johnson and Milton indicates that the aims of his account are not purely those of science as she has defined it, and that his language is neither plain nor transparent.

For Professor Stafford, science is the "recording of phenomena stripped of human history". This may have been the aim of some travellers and of most explorers, but it can hardly be said that they achieved it, unless, like Professor Stafford, we take the will for the deed. □

Harriet Guest is a Research Fellow at King's College, Cambridge.



View from above — the Battle of Fleurus, 1793-94. Taken from *Voyage into Substance*.

Separating out

C.S.G. Phillips

Contemporary Practice of Chromatography.
By Colin F. Poole and Sheila A. Schuette.
Elsevier: 1984. Pp.708. Dfl.159, $61.25.

THIS book is, in effect, a highly complex and competent review of the present state of the art in the techniques of gas chromatography, high-performance liquid chromatography and high-performance thin-layer chromatography. Little space is wasted on the history of chromatography, on the wide variety of chromatographic methods that have been devised but are now infrequently used, or on the applications of chromatography other than for analysis or preparative work. There is no reference to the various abstracting services available, or any general guide to the literature, while the wide use of computers with chromatographic instruments is only touched upon.

Where the book excels is in its attention to all the particular practical details of operating modern efficient chromatographic systems. It contains a mass of useful and critically considered information, backed by extensive bibliographies. There are two long, extremely helpful and illuminating chapters on the preparation of samples for chromatography and on "hypenated" methods (such as mass spectrometry, Fourier transform infrared and nuclear magnetic resonance) for the identification of components after they have been separated by the chromatographic column. *Contemporary Practice of Chromatography* will surely prove to be a most valued guide and source of reference for all practising chromatographers.

The beginner, however, will not find it an easy book to read; for all its many virtues, it will not serve as a general introduction to the subject. The reader is expected to know too much, technical terms are frequently used without explanation, and even when explanations are provided they are sometimes difficult to find and to follow (the index is quite inadequate). Further, the general introduction is not always convincing: for example, on page 31 alone I found four statements with which I would take issue. So although this book provides a mine of information, clearly it is one where some hard digging is required. □

C.S.G. Phillips is a Lecturer in the Department of Inorganic Chemistry, University of Oxford.

Chemical bondage

Geoffrey Wilkinson

Dictionary of Organometallic Compounds, Vols 1,2 and 3.
Executive editor J. Buckingham.
Chapman & Hall/Methuen: 1984.
Pp.3,000. £495, $990.

ALTHOUGH some organoarsenic compounds were known previously, organometallic chemistry, in which organic molecules or radicals are bound to metallic elements by metal–carbon bonds, really originated with two major discoveries in the nineteenth century. In 1827, in Copenhagen, Zeise discovered the salt $K[PtClC_1H_4]$ whose constitution as an ethylene complex, the first of the so-called "π complexes", was not fully established until the 1950s. Then in 1845, at a school in Hampshire, Frankland discovered the first true alkyl, diethylzinc. This discovery not only revealed a new area of chemistry but contributed substantially to the concept of valency. The later discovery of magnesium reagents by Grignard opened up even more possibilities as these were easier to handle as synthetic reagents than the flammable zinc compounds. The third major class is the carbon monoxide compounds, discovered by Schutzenberger and by Mond. Although "carbonyls" are not, by strict definition, organometallic, they have metal-to-carbon bonds and carbon monoxide chemistry is now so interlinked with that of the alkyls, aryls, olefins, acetylenes and related or derived compounds that it has become usual to include carbonyls under the general title.

Organometallic chemistry thus includes all manner of compounds, many of which are extremely unusual both in their structure and bonding. During the first half of this century the chemistry was dominated by organocompounds of the main elements — lithium, magnesium, mercury, silicon, phosphorus, arsenic and so on — and by utilization of magnesium and lithium alkyls and aryls in organic synthesis. Transition-metal organometallic chemistry developed only after about 1952. Carbonyl chemistry proceeded essentially independently, mostly in pre-war Germany through the work of Hieber and his school and of Fischer, Tropsch, Roelen, Reppe and others in industry where the use of transition metal compounds led to the development of new syntheses and to new industrial processes.

Over the past 35 years these two major streams have, to a large extent, merged, and organometallic chemistry today is a major field with its own journals, works of reference and textbooks. The number of papers and patents published annually has risen from a handful to thousands.

This *Dictionary* provides a list that comprises "those compounds which are in the opinion of the Specialist Editors, most typical, representative, interesting and useful or potentially useful and [presents] their properties and selected bibliography in an orderly and systematically indexed fashion". Quite a mouthful. The elements are treated in alphabetical order from silver (Ag) to zirconium. Each entry includes details of stoichiometry and molecular weight, and of some simple properties such as form, solubility, thermal and air stability, and toxicity, together with a few references to synthesis and spectra. The code-numbered compounds are also given their systematic name which many may find useful; for some of the more complicated compounds, trying to work out the name is often a substantial challenge and far more difficult than in organic chemistry. Structural diagrams are commonly provided and a useful feature is that these are repeated in the summary list at the beginning of each section. The index volume contains full names, molecular formulae and CAS registration numbers.

For workers in organometallic chemisty and catalysis, in both academic and industrial laboratories, this mammoth work will be very useful. It may be regarded as the Red Bible to supplement the now well-known Green Bible, otherwise the nine-volume *Comprehensive Organometallic Chemistry* published by Pergamon in 1982. The latter has discussions of various sorts in the descriptive text with copious data tables, while the *Dictionary* is designed to allow rapid access to limited synthetic and structural information. Readers may notice omissions; thus, out of the 145 manganese compounds no simple alkyl, such as $Mn(norbornyl)_4$ or $[Mn(CH_2SiMe_3)_2]_n$ is listed. Nevertheless, the editors' choice is pretty extensive and doubtless the promised annual supplements will fill in gaps as well as provide extensions. Expensive though it is, many institutions will have to acquire this work. □

Geoffrey Wilkinson is Sir Edward Frankland Professor of Inorganic Chemistry at Imperial College, University of London.

Starry night

Carole Stott

Maps of the Heavens.
By George Sergeant Snyder.
André Deutsch, London, Abbeville, New York: 1984. Pp.144. £19.95, $45.

THE game of joining the dots is not new. The Babylonians had such a pastime. They drew pictures by joining dots of light, or rather stars, in the sky. This ancient tradition was followed by Ptolemy whose *Almagest* (*circa* AD 150) lists over a thousand stars, grouped into 48 pictures or constellations. Today the sky is divided into an internationally accepted system of 88 constellations of which the Ptolemaic group forms the core. Many of the very earliest artistic representations are lost forever, and it is only from the advent of the printing press in the sixteenth century that we have examples in abundance. The first printed star maps were Dürer's, of 1516, and in the 300 years following, artists, mathematicians, cartographers and astronomers have all set down their own interpretations of the dots in the sky.

In *Maps of the Heavens*, George Snyder, who is in charge of maps and cartographic materials at Sotheby's New York office, looks at the evolution of the iconography of constellations in these more recent maps. The familiar 48 creatures and figures of mythology — Pegasus, Andromeda, Perseus, a lion, a dragon, dogs and bears and the rest, all of which Snyder lists — were joined by new figures. The observational work of astronomers such as Tycho Brahe, the revelations of the telescope and voyages to the Southern Hemisphere added new stars ready for forming into constellations. The stars became animals, religious figures, political symbols or tools of the sciences and arts. Some are still in use today; others, like La Lande's immortalization of his cat, Felis, or Schiller's replacement of the 12 signs of the zodiac by the 12 apostles, were short-lived.

Snyder's illustrations, which are predominantly from the sixteenth and seventeenth centuries, appear in almost chronological order. Many of them have been chosen for their beauty, not for their importance as stepping stones in the history of celestial cartography; the reader will search in vain for the influential Islamic constellations of Al Sufi, the popular and extensive *Uranographia* of Bode, and Piccolomini's star atlas of 1540 (which was not only the first printed star atlas but the first printed set of maps of the stars, as distinct from pictures of constellations). Rather, Snyder addresses himself to the colourful pageantry of celestial mapmaking.

Many of the maps betray their date or cultural and religious background through the artist's adoption of a certain artistic tradition. Here, however, the difference between the Eastern- and Western-style constellations is only hinted at as all but a handful of the 75 illustrations are from European countries. The joy of the book is the colourful and realistic photographic reproduction of the maps, which will appeal to a scientific as well as the intended, more popular audience. Each illustration is captivating in itself, while the complementary text, placed adjacent to the map, is reminiscent of an extended and authoritative catalogue entry. Consequently the book can be started and finished at a minimum of 75 different points. □

Carole Stott is Curator of Astronomy at the Old Royal Observatory at Greenwich, London, part of the National Maritime Museum.

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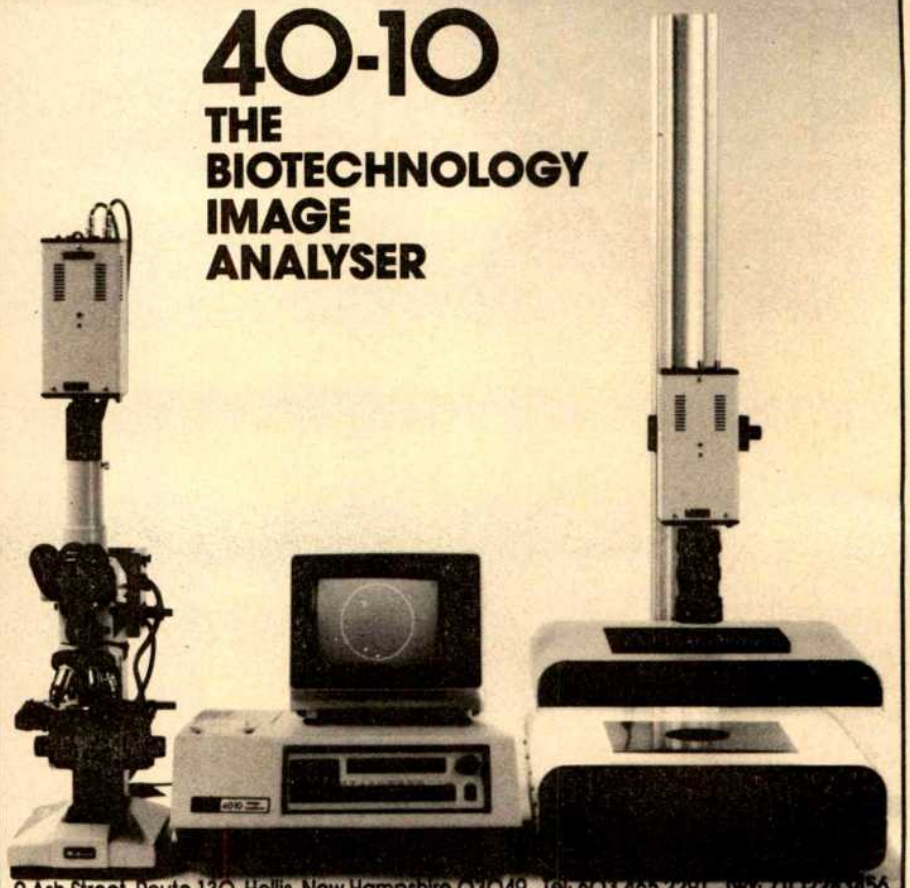
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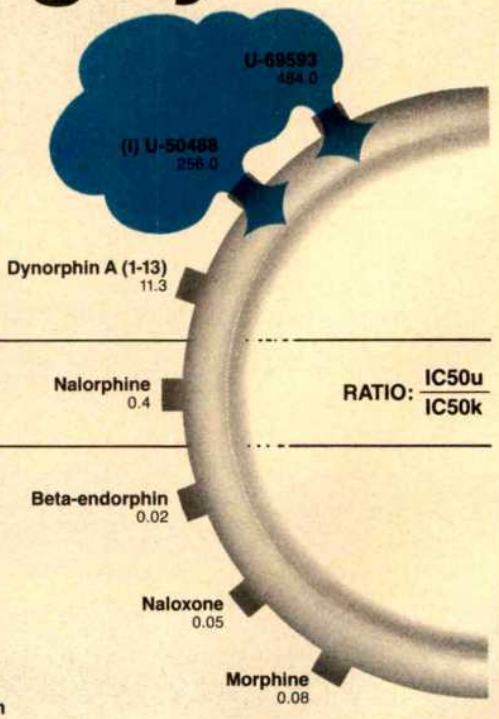
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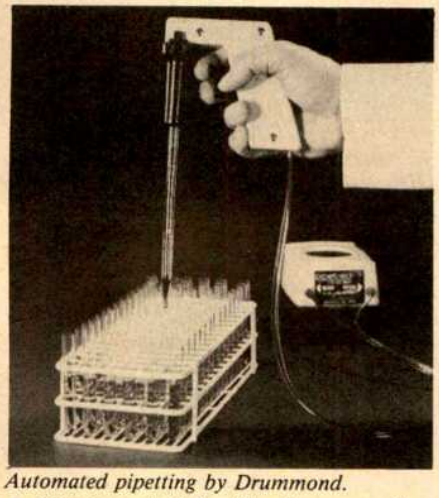
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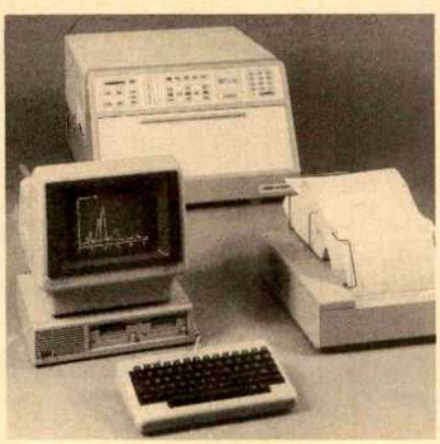
● A new storage box from Boehringer Mannheim provides a useful way of storing molecular biology enzymes. The strong plastic box is fitted with a polystyrene insert with space for 50 microcentrifuge tubes. The boxes provide a convenient means of storing enzyme stocks, buffers and DNAs at temperatures down to −80°C.
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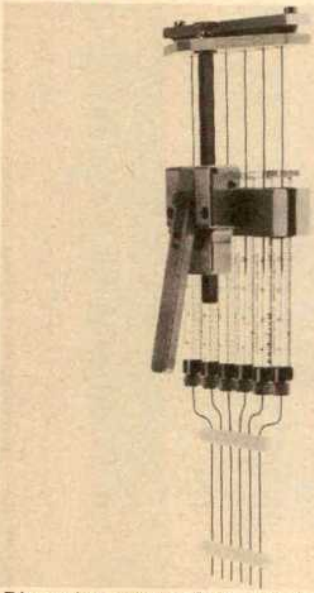


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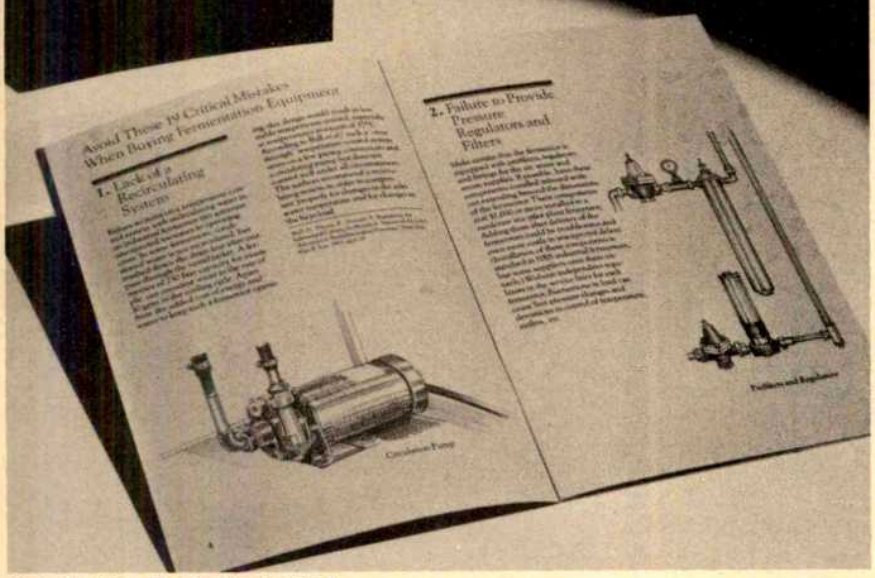
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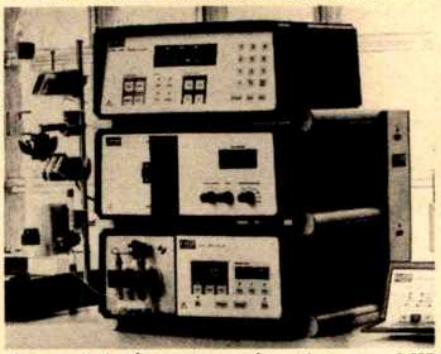


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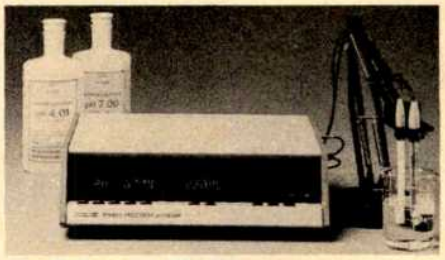
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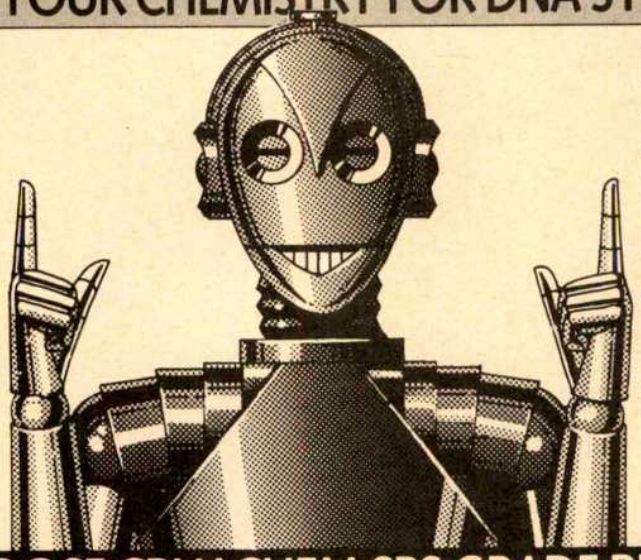
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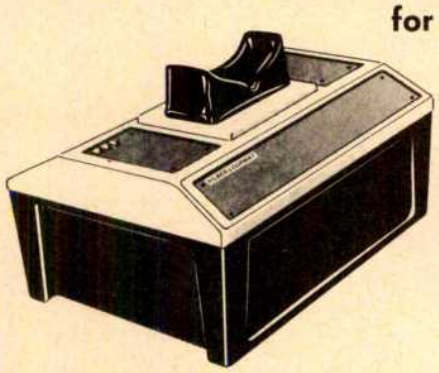
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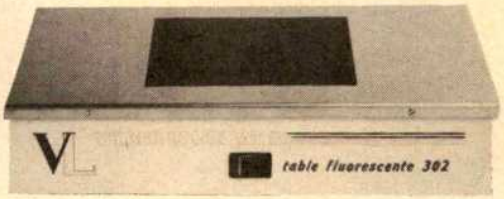
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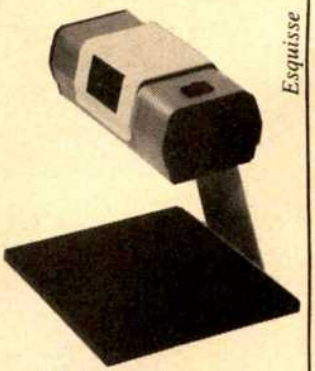
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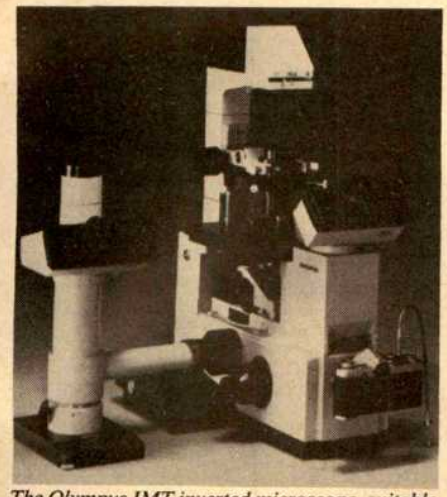
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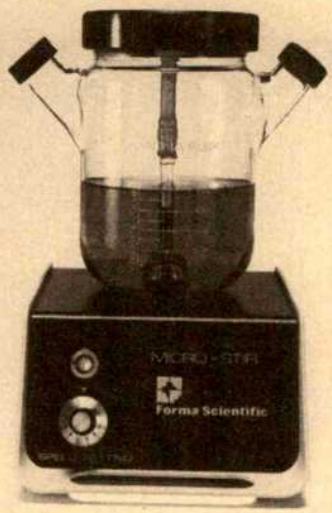
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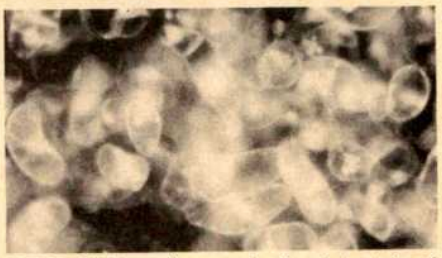
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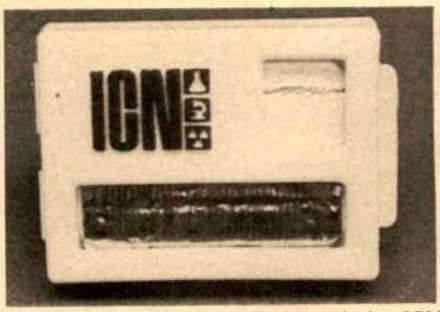
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nature

NATURE VOL. 314 28 MARCH 1985

Contrast in budget styles

India and Britain last week published taxation budgets for the year ahead. The underlying objectives are closely similar. But the Indian budget is much more imaginative.

ONE of the legacies of British rule in India is that the country's financial year begins (as in Britain) on 1 April (but there are plans to change that). So it is not simply a coincidence that the governments of both India and the United Kingdom should last week have published their plans for raising revenue during the year ahead and, in India's case, for spending it as well. (The pattern of spending in Britain has already been determined.) Curiously, the two governments have been forced by circumstances into similar strategies. Both are anxious that more should be done to make industry, and especially technically advanced industry, more prosperous. For different reasons, each government is anxious to shift the burden of raising revenue from direct taxation (of incomes and corporate profits) to indirect taxes. Each government is convinced that a well-designed budget is a means by which its domestic economy can be made productive and competitive. But this year, there can be no dispute that the government of India has made a more imaginative assault on the problems facing it that has the British government. The British government, and its Chancellor of the Exchequer, Mr Nigel Lawson, have been widely criticized for cowardliness. It seems not to have been noticed that by comparison, the government of India and its finance minister, Mr V.P. Singh, have produced a dazzling performance.

India's budget for the year ahead is the most dramatic consequence yet of Mr Rajiv Gandhi's succession as Prime Minister and of his expressed determination to make India modern. As such, his first budget should be regarded not merely as a fiscal instrument but as a strategy by means of which developing countries elsewhere might seek to drag themselves by their bootstraps into the twentieth century. For India, like many other developing countries, has nourished since independence in 1947 the notion that the development of the economy can be planned from the centre. It is not intended, even now, that this goal should be abandoned. What the new government appears, however, to have recognized is that there is a distinction between the process of channelling resources in socially desirable directions (such as the improvement of education or relief of poverty) and the attempt to control in detail the economic behaviour of the people who must be the instruments of change.

Spending

On the spending side, the Indian government plans to follow the course that has been charted in the past five years. Briefly, it will persist with the encouragement of science and technology through its technical agencies, with functions as different as the sponsorship of space research and the provision of grants for research at universities. Those similarly dependent on the British budget for research funds will be envious of the real increase planned for the coming year in India, which uniformly exceeds 10 per cent. The central government's budget will not help solve the huge problems of India's universities, most of which are the concern of state governments, but there is always a chance that the new government's good example will bring about more general enlightenment. But perhaps the most important spending decision is negative, the announcement as part of the budget package last week that government revenues will no longer be used on such a vast scale to subsidize the Indian railways. Attempts in Bengal to organize a protest boycott by railway passengers, always destined to be self-defeating, seem nevertheless to have passed off without trouble. Economic realism seems to have a logic of its own.

The revenue side of the Indian budget is more daring. But how can it matter, for the development of a technological economy, that a government should raise the funds it plans to spend in one way rather than in another? This is where Indian and the British analysts come close to agreement, believing that the burden of taxation can be an important determinant of the way in which funds are invested in innovation. That is why both governments have come to believe that it would be economically beneficial if incomes where less heavily taxed, and if the burden were shifted to what people spend. It reality, the Indian government has a further incentive to follow such a policy, for it is generally agreed that the incidence of personal taxation (which takes away roughly half the income of a person earning roughly $12,000 a year) is so sharp that it can only encourage the growth of the black economy, that based on the transfer of huge sums of cash and gold between private individuals and even corporations so as to avoid the tax net altogether. The Gandhi/Singh remedy is a substantial reduction of personal taxation, counterbalanced by increased revenue-raising from other sources, among which the largest single item so far is the increase of railway fares. The result may be to tempt back to legality some of those who at present manage to avoid paying taxes by simple dishonesty.

Realism

The government of India has taken several further steps in the direction of economic realism, which is nothing but a calculation that people should be allowed as far as possible to do what their inclinations suggest, by reducing (but not yet abolishing) the mechanism by which the government decides whether private or even partly public companies should be allowed to embark on some novel manufacturing enterprise. Ironically, even after the budget had been published at the weekend, last week's newspapers in India still carried statutorily required advertisements by Indian companies declaring their ambitions to branch out in new directions, and asking for objectors to declare themselves. The rationale for these regulations is straightforward; economic resources should not be diverted into activities that do not serve the general purpose. The problem on which India has repeatedly broken its ambitions in the past forty years is that civil servants are not much good at deciding what directions the development of industry should follow but are certainly slower than industrialists to make up their minds. And the system of industrial licensing has become one of the most flagrant foci for the corruption that has become endemic. By this attempt at letting people decide for themselves, the new Indian government may be following the present British government, but it may claim credit for having taken a gigantic step in one.

So what can be wrong with such a brave assault on convention? The snag is that the Indian government, following that of the United States, is planning deliberately to overspend its resources in the year ahead by about ten per cent, in round numbers the equivalent in rupees of $3,000 million, or between one and two per cent of President Reagan's budget deficit. Even so, such modest profligacy is a gamble. The experience of recent years has shown that the states usually manage to end up overdrawn with their bank, the central government, and that budget

deficits usually double between one April and the next. This time the government must hope that economic freedom will abate this tendency. If not, there will be inflation, the poor will feel even more poor and the momentum of development will be interrupted. Even the government acknowledges that it is betting on a favourable monsoon. We should all keep our fingers crossed.

Mr Nigel Lawson will quickly plead that a British finance minister could not dream of taking such risks; for one thing, what with the developed luxury of a welfare system, his government's spending is a much larger proportion of the national wealth. Yet Mr Lawson's problem for the past year has been very much like Mr V.P. Singh's: how to create a climate in which such wealth as there may be is directed towards productive investment, preferably in innovation. The pre-budget calculation seems to have been that a replacement of direct by indirect taxation would give saving an edge, and that it would be beneficial if the favourite media for personal savings (mortgages and pensions) could be put on an equal footing with investments in risky ventures. In the event, the British government found it lacked the courage to give such offence to its electors. Nor could it go along with the entreaties of its political opponents (not to mention a substantial section of its own political party) that the annual deficit should be deliberately increased so as to stimulate demand. (Relatively credit-worthy Britain's deficit will nevertheless be twice India's.) So Mr Lawson emerges as a piteous character, "boxed in" as he describes himself. No doubt he will be laughing if India comes a cropper, perhaps because the monsoon fails in June. But will he not be furious with himself, perhaps even as furious as his taxpayers will be with him, if India's gamble comes off when he can boast only that he did not think it the right time to make a risky budget? □

New star wars row

The private dispute at Geneva over star wars may yet be drowned by rows between allies.

MR Richard Perle, Assistant Secretary of Defense at the Pentagon, is one of the US administration's hard men, and was probably hired for that attribute, not for his skill as a diplomatist. But even so, his performance in London last week inevitably raises the question whether his operating licence should be amended.

The circumstances are very odd. Two weeks ago, Sir Geoffrey Howe, the British Foreign Secretary, had made a speech in which he made quite clear his support for research under the umbrella of the Strategic Defense Initiative and then went on to muse about the problems that would arise if ever the time came to deploy an effective defence against ballistic missiles. There would have to be negotiations with the Soviet Union about the scale and manner in which this would be done. And even when agreement had been reached, there would remain difficulties about the true measure of security an apparently leak-tight umbrella could provide, if only because the present familiarity of strategic doctrines, bizarre though they may seem, would be undermined. Sir Geoffrey went on to reflect that the difficulties he foresaw might never arise because the research programme might demonstrate that the goal is unattainable. None of this conflicts with what is understood to be the objective of the research now under way, at least since the British prime minister's pre-Christmas visit to Washington last December.

So why should Mr Perle promptly launch an attack on Sir Geoffrey at a conference held in Britain just a few days afterwards? The most charitable explanation is that he read only a bowdlerized version of Sir Geoffrey's speech, or that he was himself misquoted, or quoted out of context. Yet another explanation, so mundane that it must seem an insult to such a person as Mr Perle, is that he was jet-lagged. Yet people in his trade do not usually set about supposedly friendly statesmen with such vigour, and in public, so that some kind of explanation is essential. Otherwise, as Mr Perle must by now recognize, there is a danger that listeners will think there must be more to star wars, even now, than mere research. □

Illegality proliferates

The US administration should be more diligent in preventing nuclear proliferation

FOR an administration that has been concerned — some would say obsessed — with halting the flow of US technology to the East, the Reagan Administration has been curiously apathetic about nuclear non-proliferation. So it comes as no surprise that its apathy should set the tone for the government's law-enforcement officials responsible for federal statutes designed to prevent sensitive nuclear-weapons technology from reaching non-weapons states. There is no other explanation for the strange case of a Pakistan national arrested last year when he attempted to ship home 50 high-speed switches that just happen to be of a kind needed for building an atomic bomb. Nazir Ahmed Vaid was allowed to plead guilty to one count of violating US export laws; he received a suspended sentence and was quickly deported. Although the original indictment had made clear that the switches were sensitive nuclear technology and suggested that Vaid was working on behalf of the Pakistan government, all such references were dropped in the plea bargain. The judge who approved the plea bargain said there was no evidence that Vaid was a foreign agent; he was merely a businessman "expediting what he thought was a business deal". The federal prosecutors agreed.

An investigation by the *New York Times* has now uncovered a very different story. In fact, the government had in its hands proof that Vaid was acting directly as an agent for one S.A. Butt, who happens to be the director of supply and procurement for the Pakistan Atomic Energy Commission. Telexes from Vaid to Butt make it clear that Vaid knew it was illegal to export the switches without a licence, that he was working on "alternate ways to buy these products" after a direct approach to the sole manufacturer failed and asking what he should say the "ultimate consumer" of the products was. Butt cabled back that he should say the ultimate consumer was the research and development department of Islamabad University. The prosecutors told the *Times* reporter that they had not appreciated the significance of these telexes because they had not been able to find out who Butt was. (He had no record in the US Department of Treasury crime computer, they said.) Butt is in fact well known in arms control circles as the man who successfully procured reprocessing and enrichment technology for Pakistan from Europe in the 1970s. Had these facts come out at a trial, Vaid could have been sentenced to as much as 20 years' imprisonment for violating the Atomic Energy Act.

Precisely what Pakistan is about is anybody's guess. Since the Indian explosion in May 1974, Pakistan has encouraged speculation that it is on the way to making some kind of bomb. Most probably it has been doing its best, with help from where it could be had, people like Vaid included. On present form, dependent as it is on the United States for delivery systems, Pakistan could not realistically hope to be an independent nuclear power, but it could reasonably hope to be able to make trouble. For the past five years, the United States has been ambivalent, reckoning Pakistan an ally on Afghanistan. Until recently and perhaps still, it has fudged the nuclear issue.

It is not necessary to subscribe to conspiracy theories to see a connection between the administration's laxity on nuclear non-proliferation and the laxity of a federal prosecutor in Houston in pursuing this case vigorously. Prosecutors have a limited amount of time to devote to too many cases; they are not going to knock themselves out on cases that will not win them approval at a higher level. The administration has unmistakenly set the tone, a blend of laxity and of contempt for the law, or at least for the spirit of President Carter's Nuclear Non-Proliferation Act. The act may rashly have offended governments which believed that their obligations under the Non-Proliferation Treaty (NPT) were sufficient and may unwisely have overridden legal contracts, but is there any reason why it should not apply to countries which have scorned the NPT and which have no other claims on the United States? □

British research

Research council offers hope for astronomers

THE worst fears of the British astronomical community, that Britain might abandon observation in the Southern Hemisphere, may have been allayed by last week's meeting of the Science and Engineering Research Council (SERC). Some consider that the council was favourably disposed to the submission of its Astronomy, Space and Radio (ASR) Board for funds over the next five years, at a time when a combination of exchange rate fluctuations and pressure on the council's budget from other areas of science and from engineering have never been so acute. But the forward-look process has revealed differing perceptions of priority within the council's committees. Moreover, the long-term outlook is not yet certain, as the Advisory Board for the Research Councils (ABRC) has yet to consider the council's proposals, and some of the options involve international agreements.

By all accounts, the January meeting of the ASR board was an emotionally charged affair. Faced with the task of producing a list of priorities for the council, the board's subcommittees had thought the unthinkable and ordered their respective research programmes on strictly scientific grounds. It was the ASR's board's task to co-ordinate these shopping lists into a package for presentation to the council. In the event, there was a heated debate over proposals for ground-based optical astronomy.

Two of the most important telescopes currently at the disposal of British astronomers are the Anglo-Australian Telescope (AAT) and the UK Schmidt telescope, both in Australia. Both have proved highly successful and are generally agreed to be capable of producing world class results for several years yet. In the next few years, however, two new telescopes on La Palma in the Canary Islands — the 2.5m Isaac Newton and the 4.2m William Herschel — are due jointly to provide a first-class facility in the Northern Hemisphere.

At the January meeting of the ASR board, a certain polarization developed between those who favoured the retention of Southern Hemisphere activity those who felt that it would be better to concentrate resources at a variety of wavelengths in the Northern Hemisphere. This option would allow more collaboration, for example, between optical, infrared and radio astronomers, all of whom are strongly represented in Britain.

The prevailing view was that the Northern Hemisphere option would be the easier to sell to backers, chiefly on the grounds that it would be more easily managed. The final package that went to the council was broadly split into three. The worst-case, or lower guideline option, included studentships and grants, data processing, support for approved space projects such as the X-ray satellite ROSAT and the Comet Halley explorer Giotto, the current subscription to ESA, the European Space Agency (the 5 per cent increase recently agreed will not be paid by SERC), and the Northern Hemisphere telescopes and radars. This package would represent a 10 per cent cut in current plans.

The centre guideline option, representing the current spending level, would add support for ESA's future space programme as spelt out in its "Horizon 2000" report. The third component of the ASR board's list, and relatively low in its priorities, includes Solar System space research and Southern Hemisphere facilities.

At last week's meeting, however, the council stopped short of recommending a withdrawal from the Southern Hemisphere observatories, partly as a result of the strength of the scientific case and partly because of the international implications. Broadly speaking, the council favoured rather more than the central guideline package. One option being investigated for several of SERC's major facilities is that working time should be sold abroad to recoup costs. And, in a move that will bring weary shrugs of resignation from the staffs of the two British observatories in Sussex and at Edinburgh, yet another committee is to be set up to decide their future. This time, however, its committee is apparently to be chaired by SERC's chairman, Sir John Kingman, who is said to want this resolved before he leaves later this year.

Philip Campbell

Acid rain

US/Canada stand-off (contd)

Washington

LAST week's agreement between the United States and Canada on acid rain appears to represent no shift in the Reagan administration's policy: research, yes; controls, no. Canada, meanwhile, is accelerating its unilateral efforts to reduce emissions sources responsible for acid deposition without waiting for reciprocal action from the United States.

What last week's agreement does is to create two "special envoys" who will "examine" the acid rain issue and report within a year. The Canadians hope they will become negotiators; but the announcement suggests their role will be limited to consultation. For months, the Canadian government had made clear that acid rain would be at the top of the summit agenda. And while Canadian officials say the agreement is a hopeful sign, environmentalists have already dismissed it as a contrivance that allows Prime Minister Brian Mulroney, under considerable domestic pressure to achieve results on the acid rain problem, to claim that he did not come away completely empty-handed.

Earlier this month, however, the Canadian government was able to take credit for some more tangible steps towards acid-rain control. The federal government agreed to allocate $150 million to aid the smelting industry, Canada's major contributor to acid deposition, to reduce sulphur dioxide emissions. The provincial governments and industry will provide the remaining portion of the estimated $1,500-2,000 million. And, although it is only a minor source of the problem, Canada will as of September 1987 require NO_x emissions from cars and light trucks to match the stricter US standards; this is seen more as a political move to remove US objections that Canada was demanding US action while refusing to clean up its own house.

The Canadian government says that these steps will enable the country to reach its earlier-stated goal of reducing wet sulphur deposition to 20 kg per hectare per year in the sensitive eastern provinces — but only if the United States takes "compatible" action. Canada's reduction plans, which will cut total sulphur dioxide emissions by approximately one-half, will be completed by 1994.

Although US officials do not challenge the Canadian assertion that at least 50 per cent of acid deposition in eastern Canada is the result of emissions from US power plants (while only 10 per cent of acid deposition in the northeastern United States originates in Canada), the Reagan administration insists that more research must be done before the expense of new sulphur controls can be justified. The US argument is that not enough is known about the linearity of the atmospheric response or about how quickly lakes subjected to acid deposition actually acidify. If the lakes respond very quickly, they argue, then it is possible that virtually all susceptible lakes are already acidified, and new emission controls will make no difference.

Canada has been pressing for a 50 per cent reduction in US sulphur emissions from appropriate regions. A plan proposed by former Environmental Protection Agency administrator William Ruckelshaus but rejected by the Office of Management and Budget would have cut emissions from midwestern power plants by some 3-5 million tons, which Canada says would have been sufficient. **Stephen Budiansky**

Exhaust emissions

Europe's fiercest battle yet?

Brussels

THE political agreement to clean up car pollution in Europe reached by European governments in the long night of 20/21 March may yet be regarded as one of Europe's fiercest battles. The upshot is that there will be obligatory lead-free motor fuel throughout the Community by 1 October 1989 and, the calculation goes, a 60 per cent reduction of exhaust emissions against the yardstick of 1985. The surprise is that this solution, very much a compromise, was reached without loss of face for any member government.

The need for an agreement of some kind stems from the determination of West Germany to reduce exhaust emissions quickly, and in particular from the West German threat (uttered last year) of a unilateral introduction this July of lead-free motor fuel. In the event, West Germany went along with a package of proposals that will allow different categories of cars (classified by engine capacity) to be dealt with differently. The United Kingdom seems especially proud of having won this concession and, in particular, agreement that engines of medium capacity (between 1.4 and 2 litres) may be dealt with not by catalytic converters but by the application of novel technology as represented by "lean-burn" engines (see below).

The new standards will first make their impact generally in 1988, when new models of cars with engines above 2 litres capacity will have to conform with the new emissions standards. By 1 October of the following year, all new cars sold will have to conform. In the intermediate range, new emissions standards will apply to new models from 1 October 1991 and to new cars appearing on the roads from 1 October 1993. For smaller cars, with a capacity of less than 1.4 litres, the emissions standards are to be introduced in two stages, with a provisional standard coming into force in 1990 (for new models) and a more rigorous standard three or four years later.

Part of the uncertainty remaining about emissions standards arises because of the difficulty in defining the permissible limits of exhaust emissions from engines of different sizes. The intention, however, is that the European testing procedures will progressively be made to conform with US standards within the next few years.

One reason why last week's negotiation was successful is that West Germany eventually won the blessing of its partners for its scheme to offer financial incentives to owners of vehicles conforming with improved emissions standards. These incentives will apply from 1 July this year, but it has now been agreed that, to qualify, the exhaust emissions of small cars must be at least 15 per cent less than the transitional standards set for 1990. Moreover, the financial incentive must not exceed DM750 over a three-year period rather than the DM1,700 originally proposed by West Germany.

The underlying issue here, to which the French environment minister Huguette Bouchardeau paid most attention at last week's meeting, is that financial compensation should be "significantly less" than the cost of clean technology if it is not to contravene the Community's rules on the provision of direct subsidies for manufactured products, not to mention the environmental doctrine of "polluter pays".

The financial incentives to be offered by West Germany will include a DM0.04 per litre subsidy on lead-free motor fuel, to be introduced next week and a ten-year exemption from road-fund tax for drivers equipped for clean driving from 1 July. Friedrich Zimmermann, West German minister for the interior, admitted after the negotiating session last week that "going it alone would have meant chaos".

The United Kingdom, at the outset one of the strongest opponents of the West German initiative, also seemed content with the outcome. For cars above 2 litres engine capacity, British manufacturers seem already to have accepted that catalytic converters will be necessary. Indeed, most car models in this range depend for their economic success on export sales, for which purpose catalytic converters may already be fitted. But the United Kingdom is also pleased with the concession that lean-burn engines may be fitted to smaller vehicles as an alternative to catalytic converters.

The Community's agreement on vehicle emissions will be promulgated by means of a directive from the Commission, due to be agreed on 30 June. For the time being, diesel- powered engines and vehicles weighing more than 3.7 tonnes are not covered by emission standards, but proposals are expected by the end of the year.

Anna Lubinska

Catalytic converters or lean-burn?

Brussels

THE British arguments against catalytic converters for smaller engines are both economic and technical. It has been estimated that a catalytic converter would cost £500 at the outset, and would involve a maintenance outlay of £90 with possible replacement after four years costing £150. Allowing for fuel penalty, the cost at Community level would be 15,000 million ECU a year if catalytic converters were the only means of conforming with emissions standards.

The United Kingdom argues that a universal requirement of catalytic converters would further open up the European market to the Japanese, whose products already conform with US standards. The investment needed for the two production lines would be a burden on ailing European car industries, while the cost of catalytic converters would depress demand.

Rather than forcing the car industry down this cul de sac, the British have been arguing for time in which to develop lean-burn engines, working on the principle of altering the fuel/air ratio, thus provoking earlier combustion. The result is a fuel saving of about 15 per cent as opposed to the 5 per cent fuel penalty with catalytic converters. A prototype by Ford UK is to be demonstrated in June and could be on sale in September. The added cost to the consumer of the lean-burn engine is estimated at between £50 and £70.

The high air/fuel ratio of the lean-burn engine lowers both carbon monoxide and nitrous oxide emissions, but increases hydrocarbon emissions, for which reason an oxidation catalyst costing up to £50 would still have to be fitted.

Anna Lubinska

CERN collision

ONE of the first events taken in a streamer chamber at the European Organisation for Nuclear Research, CERN, last week, showing a proton colliding with an antiproton at 900 GeV in the centre of mass, the highest energy for such controlled collisions yet observed. Despite the complicated manoeuvres required to reach such



energies, the collider is working well, with beam lifetimes of seven hours, double what was expected, and luminosities of 3×10^{26} $cm^{-2}s^{-1}$. The plans were to introduce a gamma-ray converter around the beam pipe on Friday, to seek high multiplicity events without gamma ray production, first detected in cosmic ray observations — the Centauro events (see *Nature* 21 March, p.221).

Robert Walgate

Infant mortality

US improvement halted

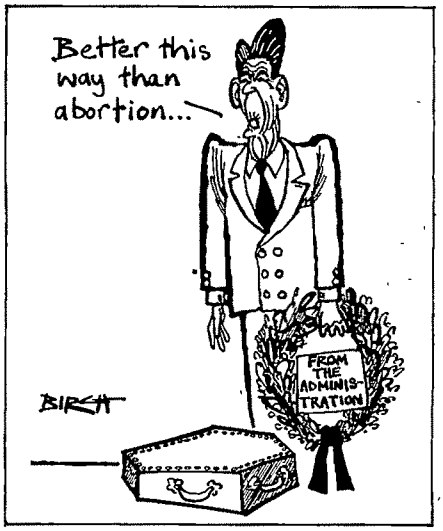
Washington

A SUDDEN halt in the improvement of US infant mortality statistics after two decades of dramatic progress is turning into a political issue for the Reagan administration as critics charge that cuts in social programmes are to blame.

From 1965 to 1981, infant deaths declined from 24.7 per 1,000 live births to 11.9. But in 1982 and 1983, the rate of improvement began to level off, to 10.9 per 1,000 in 1983, the latest year for which complete stastistics are available. Preliminary data suggest that there was virtually no improvement in 1984. And public health researchers say that the death rate has actually increased in about 10 states in the past few years.

After first suggesting that the slow-down was merely a statistical fluctuation, Dr Edward Brandt, just before leaving his position as Assistant Secretary for Health last December, acknowledged in a letter to state health officials that the problem was "worrisome". The administration has refused to comment directly on accusations that cuts in medical and food assistance programmes to the poor are to blame.

A study released last month by the Institute of Medicine (IOM) of the National Academy of Sciences lends credence to the critics' charges. It found a substantial link between inadequate prenatal health care and low birth-weight, the single greatest



risk factor in infant deaths. And the number of women visiting a physician in the first three months of pregnancy decreased in 1982 after increasing steadily during the 1970s. (The decrease was greater among black women than white women. The infant mortality rate for blacks remains twice that for whites in the United States, as it was in 1965. Black women are also about twice as likely as white women to give birth to low-weight babies.).

Those who draw a connection between the infant mortality trends and social programmes point out that the dramatic drop that began in 1965 coincided with the start of the "War on Poverty" programmes, such as federally-funded neighbourhood health centres. And according to Dr Julius Richards, who was assistant secretary of health during the Carter administration, 725,000 people have lost services at community health centres as a result of Reagan administration budget cuts. A study in Boston found that the infant mortality rate in areas of the city served by five such centres that suffered budget cuts increased by 46 per cent in 1982 over the previous years.

The group pointed to another clue for the slowdown: while mortality rates for infants less than one month old continue to fall, the rate for infants between one month and one year old may actually be increasing. Neonatal intensive care units are allowing more low-birth-weight infants to make it through the first 30 days, but more of these high-risk infants still die within the first year of life.

The experience of other countries suggests, however, that the United States can still make considerable progress — but it will take something other than advanced medical technology and intensive care units. The United States has one of the higher infant mortality rates among the industrialized nations; much of the difference, according to the IOM study, can be explained by the difference in incidence of low birth-weights. And, perhaps, more to the point, the rate of decline in infant mortality rates in the United States (until the recent slow-down) was in line with the rate of decline in other countries with substantially lower rates, according to Dr Sam Shapiro of Johns Hopkins University, which suggests that rapid progress remains a reasonable goal.

The Public Health Service is for the time being limiting its response to assisting the states in planning studies of infant deaths and identifying possible corrective steps. With the incorporation of federal service programmes into block grants to the states under the Reagan administration, it is up to the states to take those steps.

Stephen Budiansky

European synchrotron source

More horse-trading on sites

MONEY, finally, will probably decide where and when the long-awaited European Synchrotron Radiation Source (ESRS) is built. Denmark's representatives, at the latest meeting of the "Levaux committee", established by the European Science Foundation to select the ESRS site, offered 30 per cent of the capital cost if the source went to Risø, Denmark's national nuclear laboratory. Italy's delegation offered 50 per cent if it went to Trieste, a lost corner of Italy that the government dearly wishes to develop. But the French and West Germans together proposed Grenoble, near the French Alps, and offered 68 per cent (39 per cent from France and 29 per cent from West Germany). The British could have had the casting vote (preferring Grenoble), but could offer no money. And Austria and Switzerland also professed a cashless interest in the Franco-German proposal.

Meanwhile, however, the third most important figure in the French government, socialist Louis Mermaz, president of the French national assembly, has lost his seat in Grenoble as the president of the regional assembly. It was widely supposed that it was to protect Mermaz that, at the last moment, France switched its allegiance from Strasbourg (near the German border) to Grenoble. So will the French choice now revert to Strasbourg (which Germany always preferred)? "No. I don't think so. It's decided", said a French research ministry spokesman on Monday.

According to the French ministry, the Levaux committee heard site reports on Risø, Trieste and Grenoble which showed them indistinguishable on all technical counts, including vibrations and geology — whereas the handful of Grenoble sites had been in question as being close to an autoroute and the Alps. "The most dangerous vibrations come from the life of the machine itself", the French ministry claims. Thus the only distinguishing factor was money, and here France and Germany are ahead.

Nevertheless, even the Grenoble proposal comes 32 per cent short of the capital required, and France and Germany will be seeking new (paying) partners. But they will make entry as easy as possible. Thus in June, France and Germany will set up a steering committee ("provisional administrative council") to which all nations are invited "even if they can't say the exact amount they will pay". Nations that admit they cannot pay will be invited as non-voting observers.

During this year, the council will nominate a director-general, set up a scientific council and seek the cash to begin construction. If the money is found, construction will begin in the summer of 1986. Could ESRS go ahead as a purely Franco-German project (as the high-flux neutron facility, the Institut Laue-Langevin, began)? Robert Comès, whom the ministry has nominated as preliminary representatives of the Centre National de la Recherche Scientifique on ESRS, says the facility "should be European", and as French political thinking is at present strongly in favour of European initiatives and research, a Franco-German breakaway seems unlikely.

Robert Walgate

French applied science

Researchers help but stay put

FRENCH scientists are making an effort to link up with industry — but are not prepared to quit their laboratories for a year or two to work with an industrial partner. That is the picture painted by Jean-Jacques Duby, the director for applications at the Centre National de la Recherche Scientifique (CNRS), the principal French research council for basic science, at the end of his first two years in the job.

According to Duby, his new Directorate (the Direction de la Valorisation et des Applications de la Recherche, DVAR) has helped CNRS nearly double the number of new patent applications each year by CNRS groups from 71 in 1982 to 134 in 1984, while contracts with industry have tripled in value from FF 10 million (£1 million) in 1982 to FF 31 million (£3 million) last year.

Other indicators of the application of CNRS science also show improvements, such as a doubling in consultancies (to 157). But Duby's *bête noire* is lack of mobility — with only 41 CNRS staff out of a total of over 6,000 making even a temporary move to industry in 1984, a negligible improvement on the 37 in 1982. A new contract of employment for CNRS researchers, which came into effect last year, was intended to increase mobility, by giving staff civil servant status (thus guaranteeing employment and pension rights whatever moves a person might make), but clearly it has had little effect so far.

Moves in the other direction, out of industry and into research, are also rare. Duby launched a new scheme last year to grant "associate directorships of research" to industrialists wishing to spend time in CNRS laboratories. Just six such positions are about to be announced. The French, whose economy is still more agricultural and land-based than most in Europe, like to stick to their territory.

Nevertheless, there has been at least a psychological change in the relationship between French scientists and industry. The year before Duby came to CNRS, a survey of decision-makers showed that the CNRS profile had fallen "very low". But "now we even get phone calls from the big companies", says Duby. This is a "drastic improvement" on the past. But the smaller companies are still largely unaware of CNRS, and even the big companies are very wary "at risk point", when they must decide whether to invest money with CNRS in a project. "We lack venture or any other capital in France", says Duby. Another problem is that French law centres on institutions rather than contracts, so new ventures tend to require new institutions.

Despite these obstacles, Duby's DVAR has been trying every possible method of making a profit out of CNRS science, often with success. Some FF 2.8 million (£280,000) was redistributed to 54 CNRS laboratories last year from profits from patents alone. And the organization has created companies — under its new constitution, it is free to invest and make profits — such as MIDIROBOTS, a robotics company in the south of France.

Interest from industry is as great in fundamental physics as in the engineering sciences, Duby says. Organic chemistry and pharmaceuticals (where the companies have money to spend) lead the field, followed by materials science. Interest in electronics, data processing and mathematics has been "disappointing", although Duby had expected demand for work on systems and modelling.

CNRS is also putting a lot of effort into biotechnology, although this faces two major problems: on the research side, groups are often controlled by an old guard of fundamentalists, little interested in application; while on the industrial side, the big companies such as Rhône-Poulenc have rigid, seven-year research programmes into which, paradoxically, it is difficult to fit new ideas.

Duby and his DVAR are also working hard with:

- LABINFO, a database on French research and researchers, now covering 5,500 laboratories in CNRS, universities and other research councils, through which industry can trace who is working on what.
- The CNRS industrial relations committee (CRIN), whose twenty working groups discuss industrial problems and research solutions, and make proposals for research to CNRS.
- Framework contracts with companies, which cover matters such as potential patents rights, exchange of staff and payments, forming an "umbrella" under which CNRS laboratories can more rapidly reach agreement with the company on a detailed project. These contracts are being increasingly "regionalized", being signed and managed by local branches of CNRS management.
- Publicity: CNRS has been holding "industrial days" in the regions, such as one on lasers in Grenoble and one on biotechnology in Paris, and has even established an information centre at Orly airport south of Paris.
- Co-financing of applied research with the national association for the application of research, ANVAR, which works mostly with small and medium industry: this brought CNRS laboratories an extra FF 25 million (£2.5 million) in 1984.

But Duby, ever energetic, says CNRS has much more to do. The figures for CNRS/industry cooperation are simultaneously "optimistic and derisory". They are optimistic in that they are growing; derisory in that they remain too small.

Two thirds of CNRS laboratories still have no link at all with industry, and those links are with only 1,000 out of 50,000 French businesses. ANVAR, by contrast, has 5,000 such links. It is a target Duby will be aiming for.

Robert Walgate

UK higher education

Modest extra for high-tech

AN injection of £43 million into selected British universities over the next three years was announced last week by Sir Keith Joseph, Secretary of State for Education and Science (see *Nature* 7 March, p.3). The intention is that the extra money will help to bridge the expected shortfall of graduates available to the high-technology and engineering industries, particularly those involved in information technology, electronics and software. The sum to be disposed by the University Grants Committee (UGC) will be £11 million in 1985-86 and £16 million in each of the next two years.

This injection may be described as a "third time lucky" attempt by Sir Keith to fulfil an election promise to increase spending on such training. Originally, it was intended that funds would be switched within the budget of the Department of Education and Science (DES) from the arts and humanities to science and engineering. It was realized, however, that this would penalize the arts disproportionately, given the difference in unit costs between a science and an arts graduate. A second attempt to find the money was partially successful but ended in the debacle last autumn when Sir Keith was forced by a backbench revolt embarrassedly to retract a proposal that better-off parents should pay towards the tuition fees of their offspring. This diminished the extra funds for universities from £48 million to £38 million over three years.

The third attempt was supported by the Prime Minister, Mrs Margaret Thatcher, whose involvement was made necessary because the scheme requires contributions from various government departments. Those who forked out were the Welsh and Scottish Offices, and the Departments of Energy and of Trade and Industry.

UGC is already considering bids from universities for 1985-86 funds. In subsequent years, polytechnics will also be able to compete, which would represent further encouragement after the announcement earlier this year of an allocation of £2.5 million for 1985-86 for science and technology research. Mrs Thatcher has now written to some of the industrialists whose concern over future skilled manpower shortages helped to stimulate the new funds to ask for their views on the allocation of the money available and on the prospects for more direct support for university research.

Philip Campbell

Soviet academy

Winds of change discerned

THE general meeting of the Soviet Academy of Sciences, which opened only three days after the accession of Mr Mikhail Gorbachev, was quick to respond to the tone of the new regime. In his opening speech, the president of the academy, Anatolii P. Aleksandrov, put special emphasis on Mr Gorbachev's remarks at the Central Committee plenum urging all Soviet scientists to "more active involvement in the solution of important economic, public health and cultural tasks".

Such exhortations are not, of course, new. For the past two decades, every party directive on science has urged closer ties between research and production. This time, however, there was a note of urgency not always apparent at academy meetings. In a rare moment of self-criticism (relayed, incidentally, by Moscow radio), Aleksandrov noted that since the 26th Party Congress in 1981, "many scientific developments which seemed to be economically productive, important and accessible to our industry, were made use of only after a long delay".

The lag in implementing research results in Soviet production is a well-established fact of Soviet life and seems to be endemic in the central planning system, which makes no provision for the non-productive weeks or months necessary for modernizing plants or installing new equipment. The traditional scapegoat, however, is the planner or the factory manager; for scientists to accept at least part of the responsibility is, to say the least, unusual.

Whatever the explanations, Aleksandrov went on to say, Soviet scientists must now apply their efforts to finalizing the plans for the coming five years. Although the "basic directions" have already been decided by the academy and the State Committee for Science and Technology, "certain revisions are required".

What these "revisions" will entail is not too difficult to guess. This year, Konstantin Frolov, a specialist in mechanical engineering, has been elected as an additional vice-president. Each academy vice-president is responsible for a major field of research within the academy institutes; Petr Fedoseev for social sciences, Yurii Ovchinnikov for chemical and biological sciences, Evgenii Velikhov for physical and mathematical sciences, Aleksandr Yanshin for Earth sciences and so on. Although all these sectors have their applied and technological aspects, the election of Frolov to the vice-presidency suggests a policy change that would restore to the academy the technology institutes of which it was shorn in the reforms of the early 1960s.

Another field not often stressed at academy meetings is that of military research but, at a special session devoted to the 40th anniversary of the end of the Second World War in Europe, the contribution of Soviet scientists to the allied war effort was much lauded. Then, Aleksandrov continued, the level of development of Soviet science still directly influences the country's defence potential, countering "the military superiority of the forces of imperialism". The academy went on to adopt a resolution calling on "scientists of the world" to ensure that atomic research is directed towards peaceful purposes only and that space technology is used not for "star wars but to support the cause of peace and progress".

For the most part, however, the academy meeting ran true to form. Reports were presented, foreign contacts noted (28,500 Soviet scientists posted to socialist countries during the past five years) and the "complexities" of relationships with the United States over the past five years deplored. A few major innovations were mentioned almost in passing, in particular the establishment of a centre for public opinion research at the Institute of Sociological Research. Some difficulties were glossed over. Academic secretary Georgii Skryabin correctly reported the "successful research" into computer software but said nothing of the difficulties in training computer engineers recently noted in *Izvestiya* by Vyacheslav Elutin, the All-Union Minister of Higher and Secondary Specialized Education.

Perhaps, however, the most significant tribute to the new Gorbachev era came in the TASS announcement that, as expected, Aleksandrov had been re-elected president of the academy. Leading Soviet scientists are, in general, as remote from the public as are political leaders and, at 82 and in failing health, Aleksandrov has been more remote than most. Almost ten years after his first election to the post, TASS has nevertheless decided to give him something more of a public image, Aleksandrov's career was "a flurry of brilliant ideas and decisions". "He is cheerful and sociable", TASS went on, "likes a good joke and a hearty laugh. But he is furious when he sees injustice".

Vera Rich

Soviet geneticist

Smuggling charge dropped

DR David Goldfarb, the Moscow Jewish molecular geneticist, who last year was accused of trying to smuggle Soviet "national security material" — bacteriological strains — to the West, has been informed by the KGB that the smuggling charge against him has been dropped. He is now threatened, however, with prosecution for advocating a Western boycott on his behalf.

Dr Goldfarb, whose emigration visa for Israel was apparently obtained by supporters in the Academy of Sciences bypassing the normal emigration and security channels, had that visa rescinded last April, a few days before he was due to leave. His research notes, personal papers and strains were confiscated and he was informed that the strains were the property of the Soviet Union. In fact, all the strains involved had originally been obtained from laboratories in the United Kingdom or the United States, either directly or through the good offices of Wladyslaw Kunicki-Goldfinger, or of the Polish Academy of Sciences.

Recently a group of biologists from the United States and Europe proposed a moratorium on the sending of strains to the Soviet Union until Dr Goldfarb's materials were returned to him and he was issued a new exit visa. During his recent conversation with KGB officer Gusev, Dr Goldfarb was confronted with a copy of a letter he had written to Professor Elie Wollman of the Institut Pasteur in Paris, supporting the idea of the moratorium. Gusev said that the letter might constitute a case of "anti-Soviet propaganda" and made Dr Goldfarb sign a document to that effect. The document, together with the letter, will be forwarded to the prosecutor's office, in order to determine whether any further action is necessary. When Dr Goldfarb asked how Gusev had got hold of the letter, he was told that "someone picked it up in the street". Gusev added that, in any case, the moratorium would not be effective, since the Academy of Sciences said that only a few letters of protest had been received from the West".

Gusev then returned to Dr Goldfarb all the confiscated written materials (but not the strains), and said that the smuggling charges had been dropped. According to a representative of the New York-based Helsinki Watch Committee, this is the first known incident of the KGB returning confiscated material, or informing a suspect that the charges against him had been dropped.

In a telephone conversation with his son in New York, Dr Goldfarb said that so far it is unclear whether the return of the material indicates that he will be allowed to emigrate, or whether it merely marks a change in tactics by the KGB in response to Western reaction to the "strains affair". The Goldfarb case was raised during the negotiations last January about the possible resumption of the bilateral exchange agreement between the Soviet Academy of Science and the US National Academy of Sciences. According to Professor Alex Rich, a member of the United States team, Dr Yurii Ovchinnikov, a vice-president of the Soviet Academy said: "Of course this strain thing is not serious and we are trying to clear it up. But there is not much that we can do as it is in the hands of the KGB".

Vera Rich

UK–Japan exchange

Go East young man

Tokyo

GIVEN the near-impossibility of finding any kind of academic post, most young UK scientists would find it hard to believe there is a source of extraordinarily generous post-doctoral fellowships that never attracts enough applicants. But, according to new figures from the fellowship scheme operated by the Japanese Society for the Promotion of Science and the Royal Society of London, this is exactly the case.

The fellowships are available to support study in Japan's universities, many of which now boast enviable facilities, and would enable a foreigner to live in comparative luxury. A generous tax-free monthly stipend of nearly £1,000 (Y270,000) is provided, along with a monthly housing allowance of up to £350 (Y100,000) which would cover most of the rent of a reasonably spacious apartment in Tokyo, and to add icing on the cake there is an annual allowance of a further £350 (Y100,000). All of this is topped off with a round-trip airfare from Britain to Japan. But despite these incentives, only 32 of the 60 awards, which are for one or two years, have been taken up in the first six years of operation of the scheme: scarcely 50 per cent of the posts have been filled.

So what has gone wrong? Britain's figures make a poor showing when compared with identical schemes operated in West Germany and France. In these countries, all but one of the available fellowships have been taken up, and in 1983, for example, there were 35 qualified applicants for the eight fellowships open to French scientists.

Royal Society officials say the lack of applicants is due to the extremely tight situation for academic posts in Britain — few young scientists are willing to risk leaving Britain for the Far East for one or two years; they prefer to be on the spot for any "new blood" openings that might come up. But the employment situation is surely no better in West Germany and France.

Part of the problem may lie in fear of the unknown — language barriers and apprehension about the state of scientific research in Japan may deter potential applicants. It is true that any foreign scientist coming to Japan must accept that he or she does so as a "guest". On the plus side this means that a visiting scientist need not follow the gruelling work schedule of his Japanese counterparts, although some attempt to conform is expected — young Japanese scientists typically work 12 hours a day six or seven days a week. On the negative side, permanent posts in Japanese universities are virtually closed to foreigners; in 1982, government regulations were changed to allow the appointment of foreign nationals to university staffs in spite of their status as civil servants, but only two foreigners have so far been appointed as permanent members of staff in Japanese national universities. But British scientists who have ventured to come to Japan under the fellowship scheme have not regretted the move, according to John Richards, science officer of the British Council in Tokyo.

Maybe the real problem is that information about the scheme has not been widely enough disseminated in Britain. In France and West Germany, several organizations are involved in the selection procedure. In France, for example, many scientific organizations (such as CNRS and INSERM) and two ministries have representatives on the selection committee, and perhaps this more broadly based involvement of scientific and government bodies helps to draw in more applicants.

Those who criticize Japan for putting up barriers should recognize that here is a situation in which Japan has opened its doors to foreigners but few are taking the opportunity to walk in.

David Swinbanks

Asbestos

Claims centre in sight

Boston

IN the face of mounting legal costs and jammed court dockets, a group of asbestos manufacturers and insurance companies is setting up an out-of-court processing centre to handle the claims of the thousands of workers who claim to have been injured by exposure to asbestos.

After two years of negotiations, the Asbestos Claims Facility, to be based in Boston with a branch in San Francisco, will be born officially on 29 May. So far, 33 manufacturers — including the Manville Corporation, which filed bankruptcy three years ago in anticipation of financially-crippling liability judgements — and 22 insurance companies have agreed to participate.

More than 23,000 cases are now pending in state and federal courts, with about 500 new suits filed each month. A Rand Corporation study estimates that manufacturers and their insurers have already spent $1,000 million to settle asbestos-injury claims over the past ten years, with the companies' and plantiffs' legal expenses consuming 61 per cent of that total; legal expenses were threatening to escalate even further as the manufacturers and insurers began suing each other to determine who was responsible for paying off the successful plaintiffs. In joining the Asbestos Claims Facility, the companies have agreed to drop those battles. The insurance companies will provide the first several hundred million dollars (the exact amount is not yet certain) to pay off claims; if the insurance funds are exhausted, the manufacturers will shoulder the remaining financial burden.

Workers who choose to bring their claims to the facility will have to submit certain standard information, including employment and medical records. They will have to establish that they have suffered asbestos-related injury, such as asbestosis, and that they were exposed to asbestos-containing products made by at least one of the participating manufacturers. Facility staff will then try to negotiate a settlement. Failing that, the next step will be an offer to enter into mediation — binding or nonbinding — with a neutral third party. If an agreement still cannot be reached, the worker will be free to take the case to court. The facility will provide legal defence on behalf of all the companies in those cases.

Claimants, who were consulted by the companies in planning the facility, stand to gain by having their cases heard much more quickly than is now the case and by lower legal costs.

Thomas Henderson, a Pittsburgh lawyer whose firm represents 500 plaintiffs, says he is cautiously optimistic.

The Reagan Administration, which has resisted calls for government intervention in asbestos cases, has been more lavish with its praise. The establishment of the facility, says Office of Management and Budget general counsel Michael Horowitz, "demonstrates the ability of the private sector to resolve serious national problems without reliance on the government to dictate and enforce its own agenda".

Tom Burroughs

Universities clouded

THE most gloomy statement yet of the prospects for British universities was uttered last Friday by the person best qualified to judge, the chairman of the University Grants Committee (UGC), Sir Peter Swinnerton-Dyer. In a statement to a joint meeting of UGC with the Committee of Vice-Chancellors and Principals, he said that the failure of Sir Keith Joseph to refer to UGC's advice on higher education last November in a recent open letter "must be a bad sign" and a proof that the advice had been rejected.

In money terms, Swinnerton-Dyer said, the British government was expecting the universities to accommodate budget erosion of 0.5 per cent a year while asking them "to do new things". But, he guessed, the new system of cash limits would amount to a cut of 2 per cent a year. At this rate, he said, it would be necessary either that university departments or that whole universities should be closed. □

Whales

UN scientist takes a hand

WHALES and other marine mammals have found a new (objective) champion — the Yugoslav marine scientist Stjepan Keckes, for long director of the United Nations' "regional seas" programme. That programme, one of the most effective of the United Nations Environment Programme (UNEP), is now to extend its work from environmental protection to a study of fisheries and marine mammals.

Thus earlier this month, Keckes convened a meeting in Geneva of all agencies supporting marine mammal research, and had armed himself with a list of 36 research projects already mounted at a cost of $6 million and of 30 new projects, potential claimants on UNEP, which has $390,000 to spend on marine mammals this year. The environmentalist group Greenpeace proposed a project to clean up an island fouled by nets where the rare Hawaiian monk seal breeds, but most proposals were for research.

Research is sorely needed, according to British fisheries scientist Sydney Holt, who represents the Indian Ocean islands of the Seychelles on the scientific committee of the International Whaling Commission (IWC). IWC, which is a group of whaling nations and of others opposed to whaling, has proposed a ban on whaling from next year. Holt supports the ban, but says that IWC whale population models, on which catch quotas are based, are "not worth the computer tape they are stored on". In the UNEP regional seas magazine, *Siren*, he says that the survival rate of juvenile whales is a key factor in calculations but that it cannot yet be measured. Thus the models simply assume a figure, which remains constant as a function of population and population density. "In other words, we ignore it ... all our calculations are based on models in which we ignore changes in nearly every important factor."

Holt claims, for example, that there is no certainty that right whales are increasing in the Southern Ocean, as has been claimed. And humpbacks seemed to be increasing rapidly in the North-West Atlantic only because a lot of them were seen on the coast and in fishermen's nets. "But the whales were coming inshore for food because the offshore fisheries were depleted."

Keckes believes his research programme will breathe life into the floundering "global action plan" for marine mammals which has been planned over seven years by UNEP and the Food and Agriculture Organization. One objective is to help coordinate the work of bodies such as the World Wildlife Fund and the International Union for the Conservation of Nature. Governments are to meet on the issue in Nairobi next month. **Robert Walgate**

European telecommunications

Commission plans ahead

Brussels

THE European Commission has announced plans for the preparatory phase of a major research and development programme to modernize European telecommunications. Known as Research in Advanced Communications in Europe (RACE), the programme will be considered by Community research ministers at their meeting on 4 June.

Over the years, research in telecommunications has been a central part of the Community's industrial strategy. In the late 1960s, for example, a commission under Dr Pierre Aigrain singled out telecommunications as a fruitful field for collaboration but was unable to devise a workable programme. More recently, however, the Commission has been able to develop common specifications for equipment, plans for the coordination of licensing procedures and, last October, partial liberalization of European markets for equipment (see *Nature* **311**, 697; 1984).

The new programme has been put together by the Commission with the advice of national experts drawn from industry and academic life. The first objective of the preparatory phase will be to pave the way for the infrastructure needed both to support existing services and to develop new services for voice, data and video transmission. The Commission intends that there should be close collaboration between the RACE project and the Community's advanced information technology research programme, known as Esprit.

Between 1986 and 1991, work under the new programme will concentrate on integrated broad-band communications systems, when there will be some precompetitive development of equipment and services. The Commission also intends continuing the development of international standards in telecommunications.

If the first phase of the programme is agreed, the Commission would push on with a second phase between 1991 and 1996 and would then expect to invest at least 150,000 million European Currency Units (ECU) in advanced telecommunications equipment including colour telecopiers, newspaper transmission, voice-driven telecopiers and mobile videophones.

Large though this estimate may be, the Commission points out that it is small compared with the size of the telecommunications market, which is expected to amount to $500 million a year by the turn of the century. Europe is estimated to invest 20 per cent of total expenditure on telecommunications equipment, but the Commission hopes that its programme will help Europe to break into foreign markets on a substantial scale by the next century.

Meanwhile, the Commission is planning to hold a second demonstration of videoconferencing techniques in Europe in June this year and hopes that by May it will have developed a single standard for second-generation mobile telephones to replace the three standards now in use.

Anna Lubinska

European biotechnology

Brussels

THE governments of the European Community have now given the formal go-ahead for a four-year programme of research and training in biotechnology. As in other fields, the Community hopes to encourage transnational cooperation between academics and industrial interests in the ten member states.

Belatedly, 55 million European Currency Units (ECU) has been allocated for the period between 1985 and 1989, of which 9.5 million ECU are specifically earmarked for research in the collection of information, the modernization of databanks and the provision of access to existing facilities. As part of this effort, the Community hopes to improve existing collections of biological material and to develop better methods of storage and recovery.

Although the scale of support for the four-year programme falls one-third short of the sum originally sought by the Commission, the new programme is broader in scope than the first proposals, which concentrated on biomolecular engineering. While research in genetic engineering will still be supported, the new programme will also involve research on protein design as in the construction of artificial enzymes and the potential industrial applications of gene transfer. Applied research will include the development of reactors for converting waste lignocellulose into ethanol, from which the research directorate of the Commission has hitherto fought shy because of the uncompetitive prices of raw materials in Europe.

The underlying objective of the programme, according to the Commission, is to create a series of interlocking biotechnology research centres across Europe. But the cut in the research budget means that fewer contracts can be awarded, while it has been necessary to drop the study of the genetics of higher plants and the application of cell biology to the prevention of human disease, although work on the development of vaccines will continue.

Although the application of biotechnology in public health is an important aspect of the new programme, the chief focus of the research to be supported will be the use of agricultural products for industrial purposes, not for eating. The hope here is to find a solution to the problem of food surpluses in the Community.

Anna Lubinska

Better safe than sorry

SIR — I accept that if Enoch Powell's Unborn Children (Protection) Bill, for which I voted, becomes law it will obstruct and divert the course of research on the physiology of fertilization and implantation. But neither the Warnock committee, nor the Royal Society evidence to it, nor the Medical Research Council comments on its report, seemed to me to take sufficient account of the pace of current research in mammalian embryology and molecular genetics. Here most of the work is done on the mouse and other animals, with much of the most important fundamental work on the control of the structure of the embryo being done on the fruit fly. Many processes of development are similar across a wide range of species.

It seems unlikely that the fruits of such research will be a sharp distinction between a well-defined range of severe genetically based abnormalities such as Down's syndrome and a "normal" genetic make-up. There seems more likely to be a continuous distribution of manifold genetic variations with structural and functional consequences with varying social acceptability.

Shall we then reject an embryo which seems likely, say, to have a tendency towards schizophrenia, without our being able to tell whether there may be a genetic association with some less detectable tendency such as intelligence or aesthetic sensitivity? Would we wish to allow individuals, or the Secretary of State, or a medical panel such choices? Yet no sooner do we have the technique of *in vitro* fertilization available than the most pressing arguments are being put forward for developments in clinical research and practice to help with many clinical and humanitarian problems by refinements in the technique and its mode of use. Within the next few years, which will be the life of the Powell bill, the problems apparently susceptible to such treatment may well extend over wide areas of human formation and behavioural tendencies, affecting most people and most of life.

Is it not wise to explore much more thoroughly the mechanisms of genetic selection and manipulation before rushing in to apply them prematurely in ill-understood ways to human embryos? Is it not wise then to allow society time to think through the legal, social, moral and religious questions raised? That is certainly the overwhelming view of the lay public to whom we are answerable in the House of Commons. Those who want a greater freedom to experiment, for entirely understandable reasons, are playing directly into the hands of forces hostile to science.

There is a danger of building up a public backlash against science as a whole that will be more damaging, even within embryology and developmental biology, than any such restrictions as those of the Powell bill. Within a fortnight of the vote on the bill, at the annual lunch of the Parliamentary and Scientific Society, Sir Keith Joseph, Secretary of State for Education and Science, was using it to take the heat off criticisms of his slaughtering of British science, sententiously lecturing the assembled scientists about their need to take moral considerations into account in their research. It is not morality that the scientists lack, but political guile.

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Yellow rain

SIR — In their detailed letter on the yellow rain controversy, Rosen *et al.*[1] confound their own case for chemical warfare in South-East Asia. Contending that "people indigenous to the area collected and turned in anything that was yellow, in spite of the fact that yellow rain attacks were rare by mid-1982", the authors discredit the principal source of eyewitness testimony and of environmental samples, including those in which they have reported the presence of tricothecene mycotoxins. One must be suspicious of the assertion that Hmong refugees once provided authentic samples and testimony from chemical warfare attacks and at some arbitrary time ceased to do so. If we hold Rosen *et al.* to their statement, native witnesses might well have dissembled in their accounts of chemical warfare and turned in unauthentic samples at any point from 1978 to the present.

The Hmong interviews on yellow rain, which Rosen *et al.* have obviously not consulted, offer numerous accounts of chemical attacks both before and after mid-1982. These interviews, conducted from 1979 to 1983 by a variety of agencies (refs 2-4 and R. Haruff, personal communication), show a persistent pattern: the warfare scenarios, including the repercussions of illness and death, vary considerably in content and scope, while the purported chemical warfare agent seen on the ground is invariably described as yellow and matches bee faeces[5]. While the interviews by no means offer unequivocal proof of chemical warfare, they do represent the combat experience of Hmong guerrillas in Laos.

Further, the interviews suggest Hmong refugee accommodation to their US patrons' interest in building a case against Soviet influence in South-East Asia. As the bee faeces theory implies, the transformation of Laotian attacks on the Hmong into the metaphor of yellow rain may have occurred early in the post-Vietnam War phase of US–Hmong relations[5], leading refugees to procure descriptively appropriate samples whose aetiology was as much a mystery to them as to Western investigators.

The complex political vulnerability of the Hmong, both in their flight from Laos and their dependence on US officials, is an integral part of the yellow rain story. If the controversy is ever to be resolved, it will require a more studious appreciation of Hmong perspective than evidenced by Rosen *et al.* in their facile repudiation of native testimony.

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1. Rosen, J D., Cohen, H , Mirocha, C J & Shiefer, H B *Nature* 313, 271-272 (1985)
2. *Use of Chemical Weapons in Southeast Asia since the Vietnam War*, Hearings before the US House of Representatives Subcommittee on Asian and Pacific Affairs, December 1979.
3. *United Nations General Assembly Report* A-37-2959, 1 December 1982
4. *An Epidemiological Investigation of Alleged CW/BW Incidents in Southeast Asia* (Surgeon General Branch, National Defense Headquarters, Ottawa, 11 August 1982)
5. Guillemin, J. *Hmong Interviews on Yellow Rain: Cultural Rules and Political Agendas in Refugee Reports* presented at the American Anthropological Association Meeting, Denver, Colorado, 21 November 1984.

Australian databank

SIR — A computer-based system (MBIS, the Molecular Biological Information Service) for use by molecular biologists within Australia, has been put on-line by the Division of Molecular Biology of the Commonwealth Scientific, Industrial and Research Organization (CSIRO). The system can be used free of charge by authorized individuals who are either directly linked to CSIRONET (CSIRO's computing network) or through a dial-up service. Dial-up Access is charged as a local call and transmission speed can be either 300 or 1,200 bytes per second.

The system is menu driven, allowing simple access to the services available. At present, nucleotide sequence databases from GenBank, the European Molecular Biology Organization and National Biomedical Research Foundation (NBRF) are on-line as well as the NBRF protein database and are updated as soon as new versions are made available.

Bibliographic information (GenBank) is also on-line. The software library can be used to retrieve, compare and/or analyse sequence data. The uploading or downloading of information is easily accomplished. Apart from programmes written at the division, software has been made available by R. Staden and M. Kanehisa.

An electronic Notice Board and separate electronic Mail System are available which we hope will aid better communications among molecular biologists in Australia.

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A new type of forest decline in Germany

from L.W. Blank

There is much concern about the state of West Germany's large forests which are threatened by an unprecedented decline. Research into its causes no longer focuses on acid rain, but on a possible interaction of increased ozone concentrations, acid mist and climatic factors.

IN the past few years a severe and rapidly increasing decline of forests has been observed in the Federal Republic of Germany (FRG). It is now estimated that half of the total forest area is damaged to a varying extent[1]. This 'new type' forest decline, as it is called, is unprecedented in severity and geographical extent. Unlike most previous localized dieback events it cannot be attributed to a known cause.

Forest decline is now widely considered to be the most important environmental problem in the FRG, attracting considerable scientific as well as public attention. Here I present a brief account of the history of the decline, its present extent and severity, and the discussion of its potential causes.

History

First reports of damage in the early 1970s did not attract much attention or cause any serious alarm. Damage was then confined to a single species (silver fir; *Abies alba*), which accounts for only 2% of the total forest area (Table 1). Furthermore, silver fir decline is not an uncommon phenomenon in Central Europe, where localized dieback events have been well documented during the last two centuries[2,3]. However, the recent fir decline occurred almost simultaneously at various locations in the south of the country including the Black Forest and the Bavarian Forest[4-6] (Fig. 1). Similar fir damage has also been observed in Italy, Switzerland, Poland and in Czechoslovakia[7-10].

Silver fir decline in the FRG increased dramatically during the hot and dry summer of 1976 and is now affecting nearly all stands. Main damage symptoms are: gradual discolouration and subsequent loss of needles, from the base to the top and from inner to outer branches; premature growth reduction and formation of a 'stork's nest' crown; branch dieback in the lower crown and formation of epicormic shoots; extention of normal wet heartwood into the sapwood at the base of the tree ('wet core'); damage to the fine root system.

Since the late 1970s damage has also been noticed on other tree species. Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and common beech (*Fagus sylvatica*), which together account for 77% of the total forest area (Table 1), have started to show signs of damage. Symptoms are similar but not identical to those reported for fir; a comprehensive list of symptoms is given by VDI[7]. Damage to Norway spruce, the dominant species in German forests where it forms 40% of forest plantations, is of particular importance, not the least because of its considerable economic value. Monitoring plots in severely affected areas of Baden-Württemberg showed a rapid increase in damage to spruce from 6% in 1981 to 94% in 1983[11]. Similar plots do not exist for other parts of the country, and reliable data on the increase of damage are thus rare.

Damage was initially restricted to higher altitudes (above 500 metres), affecting older trees more severely than younger ones. Although there still appear to be patterns of damage due to altitude and age the variability between individual trees is so great that severely affected trees can be found next to apparently healthy ones at most sites.

Present severity

To obtain a clear picture of the extent and severity of damage the Federal Minister of Food, Agriculture and Forestry initiated forest damage inventories which were carried out by the individual federal states in the summer of 1982, 1983 and 1984. Forests were assessed using a set of criteria detailing damage symptoms for all relevant species[12]. This verbal description has since 1983 been complemented by a set of colour

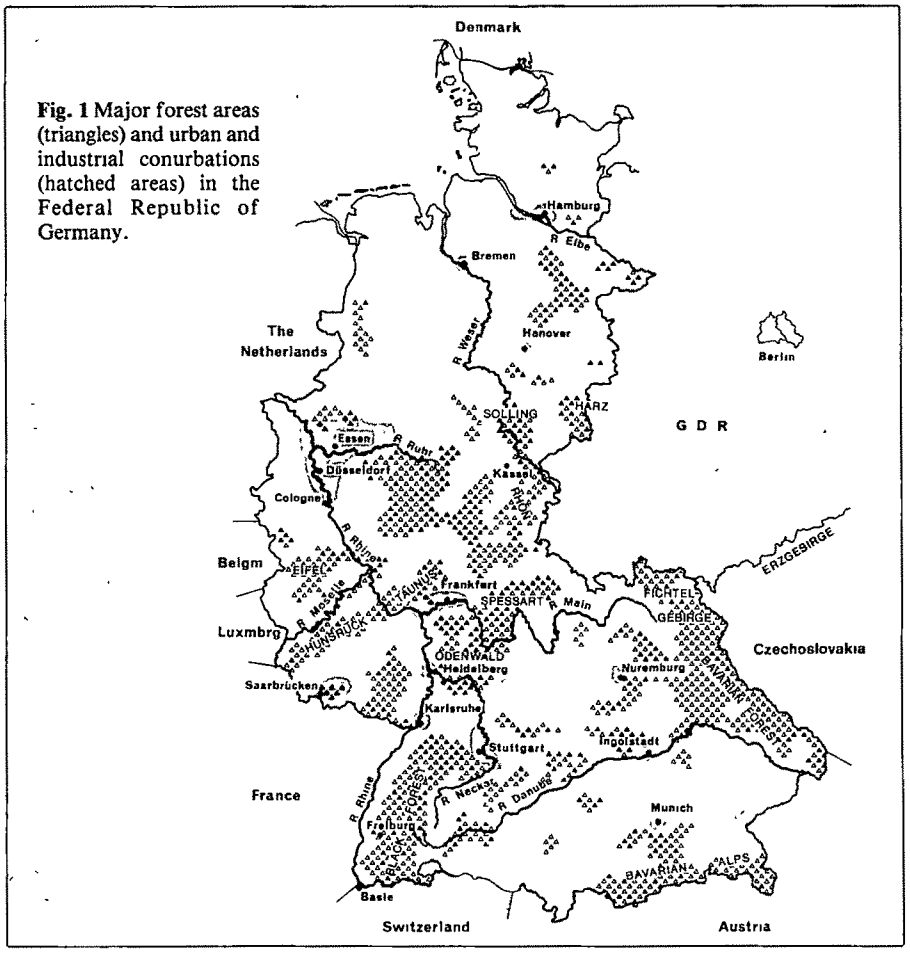


Fig. 1 Major forest areas (triangles) and urban and industrial conurbations (hatched areas) in the Federal Republic of Germany.

photographs[13]. Extent and severity of damage were classified using a system of damage categories. The initial system of three categories (category I, slightly damaged; category II, damaged; category III, severly damaged) as used in 1982 and 1983 was extended in 1984 by adding a category 0: 'without damage symptoms' and category 4: 'dead'. The main criterion for damage classification was the percentage of needle loss, which was related to damage categories. Trees with a loss of up to 10% were considered unaffected (category 0), 10-25% loss indicated slight damage (category I), 26-60% medium damage (category 2), and more than 60% loss severe damage (category 3). A second important criterion for damage classification was the discolouration of the foliage.

Before comparing the results of the three successive inventories, it is important to note that there are marked differences in their methodology and consequently in the accuracy of their damage assessment. The first survey in 1982 was largely an estimate, covering only parts of the country. The following survey in 1983, which covered the whole of the Federal Republic, used more detailed assessment criteria, and three federal states based their survey on spot-checks at assessment areas. This system was adopted on a national basis for the 1984 inventory, with a 4 by 4 km grid system determining test areas. The results obtained in these areas were extrapolated and presented as percentage damage to each tree species, to certain forest areas or to the total forest area.

Table 1 Area and species composition of forests in the FRG

Species	Area (ha × 10^6)	Proportion of total forest area (%)
Spruce	2.951	40
Pine	1.464	20
Fir	0.176	2
Beech	1.250	17
Oak	0.615	8
Others	0.950	13
Total	7.406	100

The total area of the FRG is 244,000 km^2; approximately 30% of this area is covered by forests.

Perhaps the most striking impression gained from a comparison of the three inventories is the drastic increase in the total damage forest area from 8% in 1982 to 34% in 1983 and 50% in 1984 (Table 2)[1,14]. This is mainly due to an increase in damage category I (slight damage) from 6% to 25% and now 33%, and an increase in category II (damaged) from 1.5% to 9%, and now 16%. The percentage of severely damaged and dead trees has risen only slightly from 0.5% in 1982 to 1.5% in 1984. Coniferous trees are generally more affected than deciduous species, but damage to beech and oak has risen sharply since 1983 (Table 3). Although damage can now be found all over the country, the southern states of Bavaria and Baden-Württemberg still appear to suffer most.

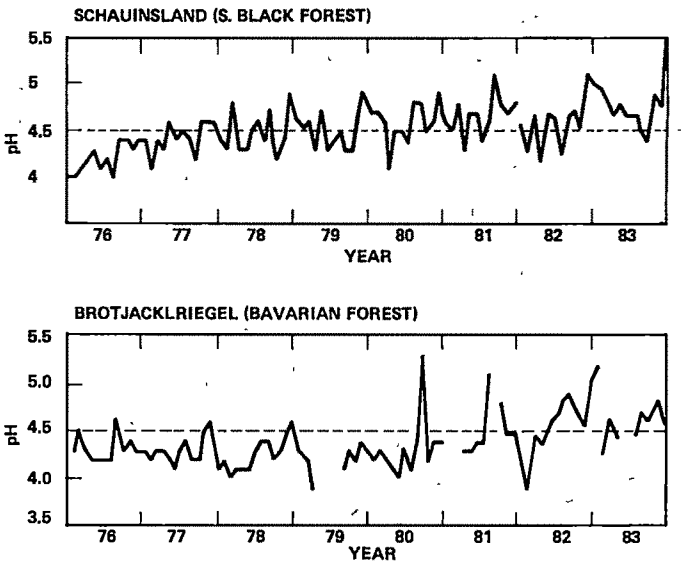


Fig. 2 Acidity of wet deposition (monthly means) in the Black Forest (Schauinsland) and the Bavarian Forest (Brotjackriegel)[19].

Disputed results

The official damage data based on the inventories are, however, not undisputed. In particular, the methods of damage assessment have been heavily criticized[15,16]. The criteria used for damage classification are unspecific but damage was, at least in 1983, ascribed to air pollution as a 'major or contributing cause'. There is now also a debate as to whether needle loss is a suitable criterion for damage classification. It has been suggested that needle loss up to 20% or 25%, currently resulting in a classification as 'slightly damaged', may in fact still be within the natural variation of healthy forests. The 1984 Swedish forest damage inventory, which is based on the German surveys, takes this into account, considering a needle loss of up to 20% as normal; the results of this survey are due to be published later this year.

Despite this controversy about assessment methods, the reliability of presented data and the comparability of the three inventories it is, however, undisputed that the decline has again increased and can now probably be seen as the major environmental problem in Germany.

Potential causes

This widespread disease, now affecting a variety of species in stands with different characteristics could not be attributed to a known cause of previous dieback events. Factors such as extreme climatic conditions (such as frost, sudden changes in temperature, drought), insect or pathogen attack, unsuitable silvicultural practices (for example, selection of the wrong species or provenance), or local sources of pollution can not explain the geographical extent of damage and its sudden increase within a few years. The discussion of the potential cause or causes very soon centred around air pollutants, which are now widely believed to play a major role in the decline. 'Acid rain', a term which at the beginning of the 1980s was becoming increasingly popular in Germany (and which was often used as a synonym for air pollution in general), was initially blamed by the media[17], and it was suggested, that SO_2 emissions in particular were the cause. This generalizing hypothesis has, however, in recent years given way to a more detailed discussion in which a number of potential causes have been suggested. It is now widely believed that the decline may be a complex disease, involving a number

Table 2 "New type" forest decline in 1983 and 1984 according to damage categories

	1983		1984	
Damage category	Area (ha × 10^6)	Forest area (%)	Area (ha × 10^6)	Forest area (%)
I	1.830	24.7	2.424	32.9
II	0.647	8.7	1.163	15.8
III + IV	0.076	1.0	0.111	1.5
I + II + III + IV	2.545	34.4	3.698	50.2

Categories defined as: I, slightly damaged; II, damaged; III, severely damaged; IV, dead. Total forest area, 7.4 × 10^6 hectares.

of factors, and there is also the possibility that the widespread decline is a number of concurrent regional events with a common trigger.

Direct effect of acid

Direct effects of sulphuric and nitric acid are, at least within the scientific community, not generally considered to be primarily responsible for the damage. The rain in affected areas is not sufficiently acid to cause damage even to sensitive plants[18], and there has been no increase in rain acidity in recent years in the Southern Black Forest or in the Bavarian forest, both areas severely affected by the decline (Fig. 2)[19].

Soil acidification

The most widely accepted initial hypothesis was based on a suggestion that wet and dry acid deposition affects the soil by destroying its buffering system. This would lead to leaching of nutrients (for example Ca, Mg, K) and mobilization of toxic aluminium ions, which would damage the fine root system of the tree, eventually resulting in its death[20]. The theory proposed that soil acidification is a natural process, but has in recent years been accelerated by increased acid input due to air pollutants. This ecosystem-orientated hypothesis of a soil-mediated effect is based on a comprehensive study of nutrient cycling in the Solling Mountains[21] (North Germany; see Fig. 1), and the results were extrapolated to other areas. It has been extensively criticized[22], mainly on grounds that damage occurs to trees on a variety of soils including those in calcareous areas with characteristics markedly-different to those found in the Solling Mountains. The hypothesis cannot explain both the temporal and geographical development of the recent decline.

SO_2 and NO_x

High concentrations of SO_2 are known to cause damage to most plants including trees[23,24]. Severe and large-scale damage to forests is reported from Poland and particularly Czechoslovakia (Erzgebirge, Riesengebirge), areas under the direct influence of high SO_2 concentrations from lignite combustion.

Only a comparatively small area in the FRG next to the East German and Czech borders (Fichtelgebirge) is exposed to similarly high SO_2 levels, with mean annual concentrations between 50 and 60 $\mu g\ m^{-3}$ and episodes of much higher concentrations (up to 1,500 $\mu g\ m^{-3}$)[25]. Damage in this area has been attributed to the direct action of pollutants, and is similar to that observed in the Erzgebirge area in Czechoslavia.

Direct effects of SO_2 and NO_2 have not been proposed as the cause of damage for the majority of sites in the FRG. SO_2 levels are generally well below the limits suggested by the International Union of Forestry Research Organizations (IUFRO). They recommend a limit of 25 $\mu g\ m^{-3}$ (annual mean), 50 $\mu g\ m^{-3}$ (average over 24 hours), and 75 $\mu g\ m^{-3}$ (97.5 percentile of 30-min values during the period of growth) in higher regions of mountains and in forests at extreme sites[26]. The Schauinsland (Southern Black Forest) and the Brotjacklriegel (Bavarian Forest) sites with forest damage, are both classified as 'clean air monitoring stations' by the German environmental protection agency (UBA). Annual mean SO_2 concentrations at the two sites during 1972-1983 were between 10 and 15 $\mu g\ m^{-3}$ (Bavarian Forest) and between 4 and 8 $\mu g\ m^{-3}$ (Black Forest), and annual mean NO_2 concentrations were below 8 $\mu g\ m^{-3}$ (Bavarian Forest) and 6 $\mu g\ m^{-3}$ (Black Forest) (Fig.4)[19,27]. A first indicator that SO_2 alone was unlikely to be the primary cause of damage was the prolific growth of lichens even in severely damaged forests.

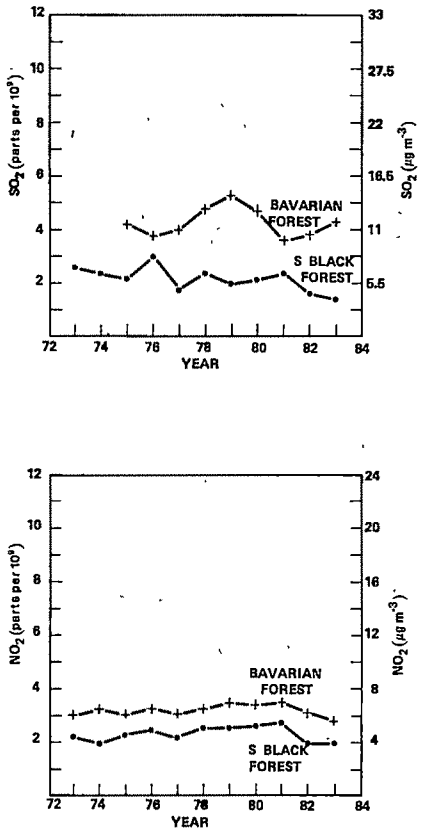


Fig. 3 Mean annual concentrations of SO_2 and NO_2 in the Black Forest (Schauinsland) and the Bavarian Forest (Brotjackriegel)[19].

Ozone

It was recently suggested that ozone (and possibly other photooxidants) may be involved in the decline[28], and the following mode of action was proposed[29]: Exposure to elevated ozone levels damages cell membranes, resulting in an increased leaching of ions by acid fog and acid rain. Ozone also causes damage to chlorophyll, and thus reduction in carbohydrate production and root growth. This reduces the ability to compensate for leached nutrients by increased uptake. A similar hypothesis includes frost as another important factor[30,31].

Ozone levels in damaged forests in the FRG are often remarkably high[29,32,33] and sometimes comparable to parts of the United States where ozone is known to have caused injury to trees[32]. Recent measurements show that daily mean ozone levels in 'clean air areas' of the Southern Black Forest (Schauinsland) and the Bavarian Forest (Brotjacklriegel) are often above 100 $\mu g\ m^{-3}$ (refs 19,29) with annual mean concentration of the order of 80-110 $\mu g\ m^{-3}$ (Black Forest) and 60-90 $\mu g\ m^{-3}$ (Bavarian Forest).

There is, however, still a paucity of data, particularly from areas with extensive forest damage. There is also a shortage of data on long-term trends in ozone concentrations — only one data set gives a clear idea of the trend in natural background ozone concentrations in Central Europe over the past 20 years. Ozone levels at Arkona on the isle of Rügen in the Baltic Sea off the German Democratic Republic, an area not affected by local air pollution, increased by 60% between 1956 and 1977 (Fig. 4)[34]. A similar trend has been reported for two other stations in forest areas of the German Democratic Republic[34], and it can be assumed for forest sites in the FRG and other neighbouring countries as well. This coincides with the steady increase of NO_x and hydrocarbon emissions from motor vehicles, which react with sunlight to produce ozone during transport over long

Table 3 Extent of 'new type' forest decline according to tree species

Species	Total area (ha × 10^6)	Affected area (%) 1983	1984
Spruce	2.950	41	51
Fir	0.178	44	59
Pine	1.463	75	87
Beech	1.262	26	50
Oak	0.612	15	43
Others	0.940	17	31

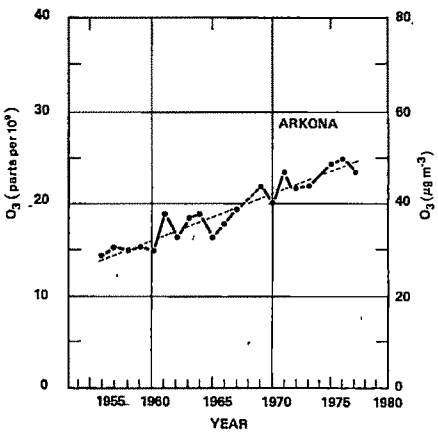


Fig. 4 Trend in mean annual ozone concentration at an unpolluted site in the German Democratic Republic (Rugen). Ozone was measured by bubbling air through KI solutions, so the data represent total oxidant. A filter was added in 1972 to remove interference from reducing gases such as SO_2 (redrawn from ref. 34).

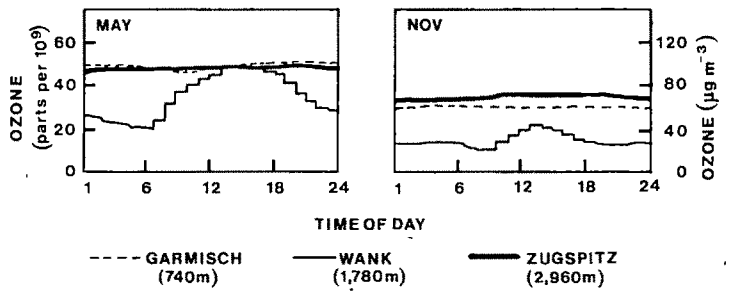


Fig. 5 Diurnal trends in ozone concentrations (1977-82) at three altitudes in the Garmisch-Partenkirchen Valley in the Bavarian Alps (Garmisch 740 m; Wank 1,780 m; Zugspitze 2,960 m). The site at Wank is close to the tree line (redrawn from ref. 35).

distances from urban areas. It has been suggested that the ozone would then affect remote areas, particularly at high altitudes, which are not under the influence of high levels of the 'classic' pollutants SO_2 and NO_x. Data from the Bavarian Alps (Garmisch-Partenkirchen) show low and steady ozone concentrations between 1977 and 1982 in a valley, and higher concentrations with a trend towards an increase at higher altitudes nearby[35]. It was at higher altitudes of remote forest areas that damage was first noticed. What is perhaps most important, in this context, is the lack of the diurnal pattern, characteristic for urban areas, at higher altitudes where concentrations appear to remain remarkably steady for long periods (Fig.5)[35].

Besides the involvement of ozone a second major factor has been suggested to interact with the oxidant; acid mist. A high incidence of fog is characteristic for higher altitudes in some damaged mountain areas in the FRG, with an annual mean of up to 226 days with periods of fog reported for a station in the Bavarian Forest[36]. Data on the frequency, the duration, and the ion-concentration of fog collected in damage areas, however, are rare.

The photo-oxidant hypothesis is at present the most widely accepted explanation for the new-type forest decline. It is, however, still largely based on circumstantial evidence and needs further supporting data from forest areas as well as experimental verification. Experimental work using or exceeding concentrations of ozone believed to be common at areas with a high incidence of damage have so far failed to produce the chlorosis which is so characteristic of the decline in Norway spruce[29,37]. There is also little known on the relative suscepability of the species concerned, and conifers, which are thought to be more resistant than deciduous species, appear to be affected first[38]. Another open question is the possibility of the quality of received radiation being involved. It cannot be ruled out that the higher proportion of ultraviolet radiation received by trees at higher altitudes could be involved in the degradation of chlorophyll in damaged needles. It will also be necessary to re-examine existing data on ambient air quality and to expand the network of air quality monitoring stations and monitored pollutants, preferably by including sites in damaged areas.

Climate

Although there appears to be a consensus amongst scientists that air pollutants play a major and probably the key role in the decline, it has also been suggested that climatic factors may be involved. The series of dry and hot years experienced during the last decade has certainly affected tree vitality and resistance to pollutants and pathogen attack. Hot summers in the continental climate have often been followed by harsh winters, with temperatures below −15°C not an uncommon event. Drastic and sudden changes in temperature as on New Years Eve of 1978 when the temperature dropped by almost 20°C within hours may be equally important.

Pathogens

As the number of trees affected by fungal infections has markedly increased over the past few years, it has been suggested that pathogens play an important role, probably as a secondary cause of decline following a lowering of the resistance by pollution or climatic factors. Rehfuess and Rodenkirchen[39] report a comparatively high incidence of fungal infection (*Lophodermium* and other fungal pathogens) from forests in Southern Germany in recent years.

There are also suggestions that the decline is an epidemiological rather than a pollutant-induced phenomenon[40]. This theory suggests, that pathogens (probably mycoplasms or rickettsia-like organisms) may be the cause of decline. This would explain the rather patchy occurrence of damage but this hypothesis is not yet supported by field or laboratory evidence.

Conclusions

The new-type decline is no longer confined to Germany. Damage has recently been reported from Switzerland (deciduous trees)[10] and Sweden (spruce)[41]. Reports in the media suggest that similar damage can also be found in Italy, Austria and the Netherlands. The situation in Eastern European countries, where large-scale direct SO_2 injury is known to occur, is difficult to assess, as there are hardly any reports on the present state of the forests.

The alarming reports on the state of Germany's forests have aroused considerable and understandable emotion, sometimes overshadowing a realistic and critical discussion of the true extent and severity of the decline, of proposed causes and potential remedial measures. There is now, however, also an increased number of research projects under way. One would hope, that laboratory as well as field work will help to unravel the causes of the decline, as all proposed explanatory hypotheses, including the currently much favoured ozone-hypotheses, are still largely based on circumstantial evidence, requiring verification under realistic conditions. It would at present certainly be premature to conclude that a conclusive explanation for this apparently complex environmental problem is available. □

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1 *Report on the 1984 Forest Damage Inventory* (Federal Ministry for Food, Agriculture and Forestry, Bonn, 1984)
2 Wachter, A *J Pl Dis protect* **85**, 361-381 (1978)
3 Malek, J *Forstw Cbl* **100**, 170-174 (1981)
4 Seitschek, O *Forstw Cbl* **100**, 138-148 (1981).
5 Wagner, F *Forstw Cbl* **100** 148-160 (1981)
6 Schroter, H *Forstw Cbl* **100**, 161-168 (1981).
7 Saurehaltige Niederschlage (Verein Deutscher Ingenieure, Dusseldorf, 1983)
8 Jarowski, A *Eur J For path* **12**, 143-159 (1982)
9 Mortondo, F & Covassi, F. *Forstw Cbl* **100**, 168-170 (1981)
10 Fluckinger, W , Fluckinger-Keller, H & Braun, S *Schweiz Z Forstwes* **135**, 389-444 (1984)
11 Schroter, H *Allg Forstzeitschr* **38**, 648-649 (1983)
12 *Instructions for the 1983 Forest Damage Inventory* (Federal Ministry for Food, Agriculture and Forestry, Bonn, 1983)
13 *Allg Forstzeitschr* **22** (Special Issue Forest Damage) (1983)
14 Lammel, R *Allg Forstzeitschr* **14/15**, 340-344 (1984)
15 Ballach, H -J & Brandt, C J *Staub-Reinhalt Luft* **43** 448-452 (1983)
16 Fuhrer, E , Halbwachs, G , Donaubauer, E , Pollanschutz, J & Stefan, K *Holz-Kurier* **39**, 13-14 (1983)
17 *Der Spiegel* 47/48/49 (1981)
18 Jacobson, J.S. *Phil Trans R Soc* **305**, 327-338 (1984).
19 Umweltbundesamt (UBA), Monatsberichte aus dem Meßnetz (1976-84)
20 Ulrich, B in *Ecological Effects of Acid Deposition*, 221-231 (National Swedish Environment Protection Board, Solna, 1983)
21 Ulrich, B , Mayer, R & Khanna, P K. *Schriften Forstl. Fak Univ Gottingen*, 58 (1979).
22 *Gesamtverband des Deutschen Steinkohlenbergbaus, Saurer Regen und Forstschaden* 2nd edn (Essen, 1983)
23 Roberts, R M , Darrall, N M. & Lane, P *Appl. Biol.* **9**, 1-142 (1983)
24 Materna, J *Prace Vulhm.* **43**, 169-177 (1973)
25 Der Rat von Sachverstandigen fur Umweltfragen, Waldschaden und Luftverunreinigungen (Kohlhammer, Stuttgart/Mainz, 1983)
26 *IUFRO News* **25** (3) (1979)
27 Umweltbundesamt (UBA), Texte 33/1982 (1982)
28 Arndt, U , Seuffert, G & Nobel, W *Staub-Reinhalt. Luft* **42**, 243-247 (1982)
29 Prinz, B , Krause, G H M & Stratmann, H *LIS-Berichte* **28** (1982)
30 Bosch, C., Pfannkuch, E , Baum, U & Rehfuess, K E *Forstw Cbl.* **102**, 167-181 (1983)
31 Rehfuess, K E *Allg Forstzeitschr* **24**, 601-610 (1983)
32 Ashmore, M R , Bell, J N B & Rutter, A J. *Forest Damage in West Germany and the Role of Ozone* (Imperial College, London, 1984)
33 Guderian, R , Tingey, D T & Rabe, R in *Umweltbundesamt Berichte 5/83* (ed Guderian, R) 205-429 (1983)
34. Warmbt, W *Zeitschr Met.* **29**, 24-31 (1978)
35 Reiter, R *Bayerisches Staatsministerium fur Landesentwicklung und Umweltfragen, Materialien* 28 (1983)
36 Noack, E M *Nationalpark Bayerischer Wald*, 5 (Bayerisches Staatministerium fur Ernahrung, Landwirtschaft und Forsten, 1979)
37 Skeffington, R A & Roberts, T M *Oceologia (Berlin)* **65**, 201-206 (1985)
38 Davis, D D & Raymond, G W *EPA Report* EPA-600/3-76-102 (1976)
39. Rehfuess, K E. & Rodenkirchen, H *Forstw Cbl* **103**, 248-262 (1984)
40 Kandler, O *Naturwiss Rundschau* **11**, 488-490 (1983)
41 Kvist, K & Barklund, P *Forstw Cbl* **103**, 74-82 (1984)

Recalculating interatomic forces

The van der Waals force between atoms and molecules is generally taken to be inversely proportional to the inverse sixth power of the distance, but positronium atoms may interact differently.

ALTHOUGH the van der Waals force has been part of classical physics for the best part of a century, since the recognition that the departure of the behaviour of real gases from the perfect gas laws points to a short-range attractive force between atoms and molecules, there is no classical explanation of the effect. Provided that the interacting species have no permanent dipole moment (when the van der Waals force is negligible), the best that can be done is to suppose that atoms and molecules are attracted to each other as a consequence of their polarizability; each becomes a little dipole under the influence of the other.

Classically, it is easy to see that this force has a short range in the sense that it will be proportional to a large power of the reciprocal distance R between the interacting entities (for the force due to an induced dipole is already proportional to $1/R^3$), but two interacting atoms which mutually induce a symmetrical pair of dipoles will be found to have a mutual energy greater than they began with, corresponding to repulsion, and which classically will not happen. That is why the van der Waals force has been regarded as essentially a quantum phenomenon at least since F. London's successful account of it half a century ago. Now, even that has been refined by means of an elegant calculation due to J.R. Manson and R.H. Ritchie (*Phys. Rev. Lett.* **54**, 785; 1985). The outcome could be interesting.

The simplest way of calculating the van der Waals force gives a reasonable result, but is now shown to be subtly too simple. For definiteness, suppose that the two interacting atoms are two hydrogen-like atoms whose nulcei are separated by a distance R. To a first approximation, the single electrons of each of them are described by a set of hydrogen-like wavefunctions centred on points separated by a distance R. The more realistic next approximation then consists in the addition to the total energy of the system of the terms representing the interaction between the pair of nuclei and the pair of electrons (both positive, denoting repulsion) and between each nucleus and the other electron (negative, for attraction).

Practitioners of wave mechanics will take this kind of problem in their stride. The extra interaction energy is supposed small compared with the interaction between each nucleus and its "own" electron, so textbook perturbation theory applies. The complete set of wavefunctions of each atom enters into some linear combination of the wavefunctions corresponding to all of its quantum states. London remarked that geometrical symmetry ensures that the first-order perturbation calculation is zero. But second-order perturbation theory yields a plausible result: an attractive force between atoms proportional to $1/R^6$.

At its simplest, perturbation theory is a way of calculating the energy of a system that differs to only a small extent from one that can be solved exactly. For the calculation of van der Waals force between two hydrogen-like atoms, for example, the behaviour of each electron under the influence of its own nucleus is exactly calculable, and the result is the familiar infinite set of discrete energy levels converging on the ionization energy of the atom. The energy of the combined system of two atoms close together is then approximately calculable from the terms representing the interaction between the atoms and the wavefunctions corresponding to the original unperturbed system, the product of two one-electron wavefunctions centred on the positions of the two nuclei separated by a specific distance.

Second-order calculations give a familiar result. The energy of the interacting system differs from that in which the interaction is ignored by an amount expressed as an infinite series in which each term represents the square of the probability that the interaction energy will induce a transition from the original state to another but weighted by the reciprocal of the energy difference between the states. Straightforward calculation along these lines recovers the London result that the energy of the combined system is decreased by an amount proportional to $1/R^6$, entirely appropriate for the short-range van der Waals force.

But the argument is incomplete. Implicit in the calculation is the assumption that the pair of interacting atoms is static, with the two nuclei fixed in space. Yet the calculation itself suggests that the interaction is responsible for an attractive force between the pair of atoms which in the absence of specified constraints, should be sufficient to make the configuration of the system time-dependent.

The circumstances resemble those familiar in the calculation of the properties of molecules, where it is customary to calculate electronic states on the assumption that the atomic nuclei are fixed in space, and then separately to calculate such things as the vibrational frequencies of the molecule, which entails allowing that the nuclei can move. The justification of this is the Born-Oppenheimer approximation, which is usually sustained by the observation that the characteristic frequencies of electronic motions are much greater than those with which atomic nuclei move. One day some lucky graduate student will find a supervisor to back sober up-to-date investigation of the circumstances under which the approximation does not apply.

Meanwhile, Manson and Ritchie have provided an intriguing example with which to begin, the van der Waals interaction between two positronium atoms, the metastable atoms consisting of a positron and an electron, the least massive of all hydrogen-like atoms. The starting-point for the calculation is a description of the unperturbed state of the system which allows that each of the interacting atoms may be in motion (so the wavefunctions are products of two atomic wavefunctions and two momentum wavefunctions, or plane waves). What Manson and Ritchie have done is to calculate the van der Waals force between two atoms whose initial states are described in this way.

First, there is a matter of interpretation, which goes back to London. The terms in the series representing the second-order perturbation energy, which are products of the probability that the interaction energy will cause transitions between electonic states, may be regarded as representing processes in which a virtual photon is allowed to excite the system in a defined way and then be emitted again, to be absorbed where it began. But, in such a process, there must be not merely a transfer of energy but of momentum — the atoms concerned must recoil. Manson and Ritchie have allowed for recoil in the calculation of the forces between positronium atoms. So the $1/R^6$ behaviour of the van der Waals force is modified, by the effects of recoil, to one that tends to $1/R^4$ at small separations between the atoms.

The chief interest in this work will be the neatness of the calculation. For the interaction between positronium atoms, the departure from $1/R^6$ behaviour should be recognizable out to a distance of four atomic units, and may thus be measurable. So should be departures from London's result for the interaction between positronium and other atoms. Meanwhile, the calculation is a telling reminder that even the best-tried algorithms should be constantly reassessed. **John Maddox**

Synthetic fuels

From lignin to coal in a year

from John Larsen

SEVERAL million years and a minor imperfection in the carbon cycle have given us coal, yet Winans and his colleagues at Argonne National Laboratory have taken less than one year to prepare a thoroughly characterized synthetic coal[1]. The material they produce is indistinguishable from the real thing by all the techniques so far applied to it and its synthesis raises many interesting questions in coal chemistry.

Coals are extraordinarily complex mixtures, which Neaval has likened to fruitcakes[2], with the various components originating from different parts of the plants from which the coals are formed. Coal's organic constituents, which range in size from methane to macromolecular gels of effectively infinite molecular weight, are mixed throughout with a variety of inorganic materials, principally clays. Determining the structure of coal has been difficult because the normal techniques of organic chemistry are not well suited to complex macromolecular mixtures.

As is usually the case, instrumental advances have been responsible for much of the recent progress in understanding coal structures. The resurgence, in semi-quantitative form, of the concept of coal as a three-dimensionally cross-linked macromolecular structure — originally developed by van Krevelen[3] — came just in time to support new and improved spectroscopic techniques[4]; the switch from grating IR instruments to Fourier-transform IR spectrometers not only added the kind of sensitivity that is so valuable with opaque samples, but also enabled spectra to be subtracted from one another, an enormous aid when working with complex mixtures. The recent availability of high-resolution nuclear magnetic resonance (NMR) spectra of solids is also being vigorously exploited and has greatly extended our ability to follow structural changes. This technique is still troubled by the 'invisibility' to NMR of many of the carbon atoms in coal, but all the indications are that visible carbon atoms are representative of the whole sample. New chemical methods, mostly better oxidative[5] and reductive[6] degradation techniques, are also contributing to structural information on coal. But these contributions have added little to our understanding of the process by which coal is formed. It is in this respect that the Argonne group's description of a convenient preparation of man-made coal is likely to be most important; already it has raised the possibility that natural coal production is a catalytic process.

The synthetic coal is produced by warming lignins (highly aromatic molecular components of woody tissue) at 150° C for a few months in the presence of twice as much montmorillonite clay, which seems to serve an acid-catalytic role. Fourier-transform IR, NMR, electron-spin resonance, gas chromatography, mass spectrometry and oxidative degredation were used to analyse the product. The changes that were observed are similar to those that occur in natural coal production. Lignin heated without clay did not produce coal, and heating cellulose (the other portion of woody tissue), with or without clay, also failed to produce coal, perhaps settling the controversy over the role of cellulose in coal formation. More surprisingly, the clays cause fatty acids to form a coal component called alginite, which is thought to derive naturally from the remains of algae that contain fatty acids, as an important lipid constituent.

The most thorough previous attempt to produce coal artificially was a thirty-year experiment initiated at the US Bureau of Mines by R.A. Friedel[7,8]. This experiment, which outlived the laboratory that started it, consisted of pyrolysis at 200°C of several possible coal precursors, including pine wood and lignite, a very young brown coal with high oxygen content. As judged by NMR, electron-spin resonance, IR spectra and elemental analysis, in only eleven years lignite was altered to a material similar to a much older coal and the pine wood formed material similar to a subbituminous coal. Interestingly, pyrolysis of pine wood at 300°C for one hour produced coal-like materials, which contrasts with Winan's observation using lignin as a starting material. According to H. Retcofsky, who is completing a study of the Bureau of Mines's samples, pyrolysis for eleven years at 200°C is roughly equivalent to one hour at 290°C.

The Argonne work raises many questions, particularly concerning the relevance of acid catalysis using large amounts of clay. Real coals often contain only a few per cent of coal clays. Is their production catalysed by clays? What is the mechanism by which acid catalysis is extended into the solid from the clay surface? Is this mixture of solids mobile? If so, will time alone allow the few per cent of clay in natural coal to convert the entire mass? What is the *p*H dependence of this acid-catalysed process? These questions will surely be addressed. The production of artificial coal will open a new era of the study of coal formation by allowing the direct experimental test of hypotheses. The preparation of isotopically-labelled coals for spectroscopic and other studies, is already underway and will be welcomed by those trying to unravel coal reactions. □

1 Hayatsu, R , McBeth, R L., Scott, R G , Botto, R. E. & Winans, R.E *Org Geochem* 6, 463 (1984)
2 Neavel, R C In *Chemistry of Coal Utilization* Suppl. Vol (ed Elliott, M.A.) Ch 3 (John Wiley, New York, 1981)
3. van Krevelen, D W *Coal* (Elsevier, New York, 1981)
4. Karr, C Jr *Analytical Methods for Coal and Coal Products* (Academic, New York, 1978).
5. Hayatsu, R., Scott, R G. & Winans, R E. in *Oxidation in Organic Chemistry* Part D (ed Trahanovsky, W. S) (Academic, New York, 1982)
6 Stock, L M in *Coal Science* Vol 1 (eds. Gorbaty, M L. Larsen, J W. & Wender, I. (Academic, New York, 1982)
7 Friedel, R A., Queiser, J. A & Retcofsky, H. L *J phys Chem* 74, 908 (1970).
8. Friedel, R A & Retcofsky, H L in *Spectrometry of Fuels* (ed Friedel, R A) Ch 5 (Plenum, New York 1970).

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Early mathematical wheelwork

Byzantine calendrical gearing

from Francis Maddison

FOUR fragments of a sundial incorporating a geared calendar (Fig. 1), inscribed in Greek and probably dating from the late fifth or the early sixth century of our era, document a gap in the history of precision gearing as yet unfilled by literary evidence. The fragments are extensively described and discussed by J.V. Field and M.T. Wright in a forthcoming paper[1]; and at an exhibition* that opens today in London's Science Museum, the fragments are set in historical relationship to the very few surviving devices with precision gearing from the last century BC to the beginning of the fourteenth century.

It may still commonly be thought that gears with precisely determined numbers of teeth were first used in mediaeval clocks, but an early discovery of underwater archaeology disproved that view. In 1901, the cargo of a ship which sank around 80 BC near the island of Antikythera in the Aegean was found to contain a corroded mass of bronze gear-wheels and fragments of bronze plates with Greek inscriptions. The late Derek de Solla Price showed that these remains had formed part of a complex calendrical computing device, a fact which fitted uneasily into the traditional view of a Hellenistic society little interested in technology[2]. The main input into the box containing the mechanism was by a crank which rotated a contrate wheel to turn a main wheel, one revolution of which represented a solar year. Outputs seem to have included a function of the Metonic cycle (using a differential), the relative positions of the Sun and Moon, and the risings and settings of certain stars throughout

*'Early Gearing' Geared Mechanisms in the Ancient and Medieval World', from 28 March 1985. Exhibition catalogue from Science Museum, London SW7 2DD, UK

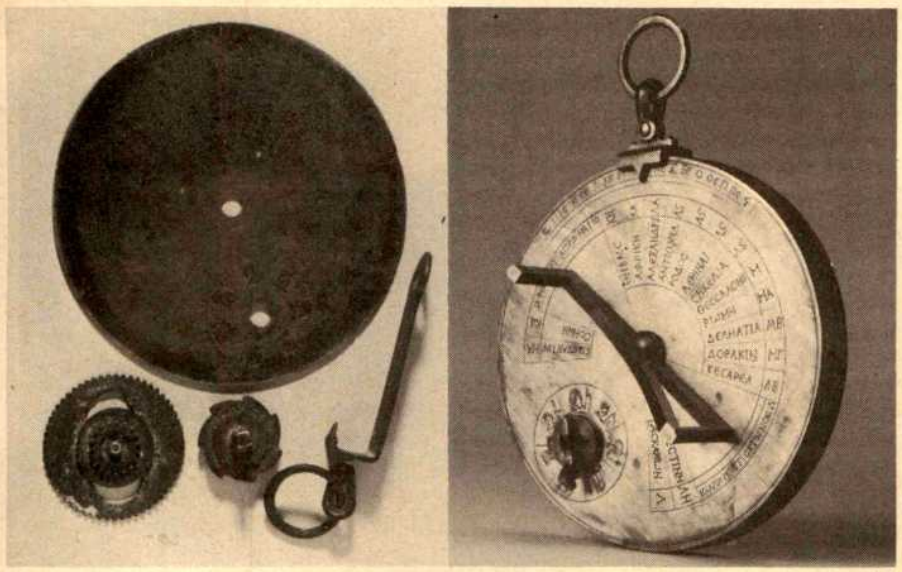


Fig. 1 The four fragments (left) and a reconstruction (right) of a Byzantine sundial-calendar with Greek inscriptions and probably made in AD 479-565. The large disk has a diameter of 135 mm. Reconstruction by M.T. Wright. (Courtesy Trustees of the Science Museum).

the year. The Science Museum exhibition includes a model of the Antikythera machine (lent by the Smithsonian Institution, Washington).

Price related the triangular-toothed gearwheels of this mechanism to a Hellenistic tradition of automata and mechanical toys, applied to an astronomical water-clock tradition, as recorded by Vitruvius around 25 BC. He pursued the tradition further, by way of Islamic interest in automata and water-clocks with opening windows, spherical weights falling into gongs, and moving human figures, to the mediaeval European astronomical clocks, with their mathematical modelling of the motions of the celestial bodies (as on the planetary clock of Giovanni de' Dondi, completed in 1364) and astrolabe dials and jacks (like the clock of 1410 on the Old Town Hall in Prague)[3-5]. This is an involved and complicated story, fascinating in its implications for motivation and cross-cultural transmission, but the gaps in the sequence are wide and the pursuit of detail a difficult task.

Chronologically, the history of mathematical gearing has been devoid of surviving artefacts or literary description from the Antikythera mechanism until about AD 1000, when the eminent Islamic scientist al-Bîrûnî wrote a treatise on the different types of astrolabe, and included an account of a geared calendar. The Science Museum exhibition includes a reproduction of al-Bîrûnî's text and an astrolabe (made in AD 1221-2 by Muhammad the son of Abû Bakr, the needle-maker of Isfahân, and borrowed from the Museum of the History of Science, Oxford) which includes just such a geared calendar (Fig. 2), though with slightly different gear-ratios. On the back-plate are apertures showing (in the Arabic alphabet, used numerically) the age of the Moon, and its phase by the rotation of a silvered and blackened disc; furthermore, the relative positions of the Sun and Moon are indicated by inlaid gold and silver points on concentric rings within a scale of the signs of the zodiac. A gear-train links the manually-turned discs giving the data, confirming al-Bîrûnî's remark, two hundred years earlier, that the wheels in his calendar were like those fitted to the backs of astrolabes.

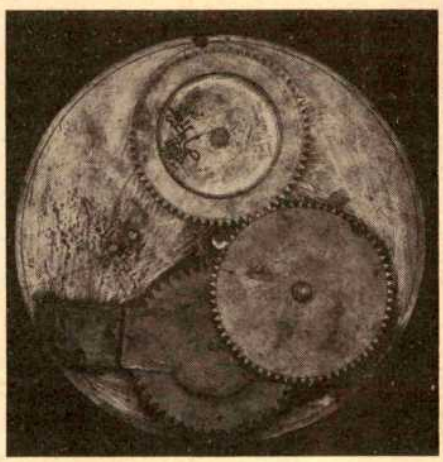
The fragments acquired by the Science Museum, regrettably without any provenance beyond the vendor's statement that they were bought in the Lebanon, fall chronologically midway between the Antikythera mechanism and the al-Bîrûnî calendar. A detailed metallurgical, technological and historical study of the fragments[1], together with a new translation and commentary on al-Bîrûnî's description of the geared calendar[6], show that the fragments were part of a geared calendar, the gear-ratios of which correspond exactly with those given by al-Bîrûnî, associated in this case not with an astrolabe but with a portable vertical disk sundial of a type well-known from late antiquity. The Greek inscriptions and the date of manufacture attributed to the fragments — before AD 650, probably AD 479-565 — indicate a source for the al-Bîrûnî calendar, and push back its origins by at least 500 years. Among the fragments is the front plate which bears a scale for adjustment to the latitude of the sundial, scales of solar declination for adjusting the gnomon (missing) which bore the hour-lines, and a table of the latitudes of various places (including Constantinople, Alexandria, Athens, Sicily, and Rome). It also has a circular scale of the days of the week (indicated by pictorial representations of the 'rulers' of the day), over which moved a pointer linked to the calendar. The back plate and some wheelwork are missing, but a ratchet survives, suggesting that the calendar mechanism might have been 'clicked on' each day, to provide the date for setting the gnomon. Field and Wright discuss the possible use of the calendrical mechanism to convert sundial readings by moonlight to solar time, and to predict eclipses. Remarkably, geared calendars of this type were still in use at the end of the sixteenth century.

Because of their age, the new fragments impose a revaluation of commonly held views of Byzantine science and technology. The exhibition of which they form a part covers an important and exciting theme — fourteen centuries of precision mathematical gearing for computation before the end of the European mediaeval period.□

1. Field, J.V. & Wright, M.T. *Annals of Science* (in the press).
2. Price, D.J. de S. *Scient. Am.* June 1959; *Gears from the Greeks* (Science History Publications, New York, 1974; reprinted from *Trans. Am. Phil. Soc.* n.s. **64**, 1; 1974.
3. Hill, D. *A History of Engineering in Classical and Medieval Times* 183 (Croom Helm, London & Sydney, 1984).
4. King, H.C. & Millburn, J.R. *Geared to the Stars. The Evolution of Planetariums, Orreries and Astronomical Clocks* Ch. 1-4 (Toronto U.P., Toronto and Hilger, Bristol, 1978).
5. Bedini, S.A. & Maddison, F.R. *Mechanical Universe. The Astrarium of Giovanni de' Dondi* (Museum of History and Technology, Washington 1966; reprinted from *Trans. Am. Phil. Soc.*, n.s. **56**, 1; 1966).
6. Hill, D.R. *Annals of Science* (in the press).

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Fig. 2 Calendrical gearing (left) and back-plate (right) of a Persian astrolabe made in AD 1221-2 by Muhammed the son of Abû Bakr, the needle-maker of Isfahân. (Courtesy Museum of the History of Science, Oxford.)





Immunogenetics

Lymphocyte development genes and immunodeficiency disease

from S. Darling and P. Goodfellow

UNLIKE AIDS, some human immunodeficiency diseases are inherited rather than acquired. Several of these genetic diseases are linked to X-chromosome loci and are responsible for catastrophic illness[1,2]. As neither the immunoglobulin genes[3] nor the T-cell receptor genes are themselves sex linked[4-6], it has been suggested that the X-linked disease loci might include regulatory genes responsible for lymphocyte development. This hypothesis is supported by two papers in this issue[7,8], which report the isolation of an X-linked sequence that is developmentally expressed in lymphocytes and is aberrantly expressed in a mouse model for X-linked immune deficiency.

CBA/N mice have an X-linked mutation, *xid* (reviewed in ref.9), which renders them incapable of producing antibodies to soluble polysaccharides. Their immunodeficiency is caused by a B-cell defect and the mice seem to lack a specific subpopulation of mature B cells. In humans, a similar defect in antibody production is present in the X-linked Wiskott-Aldrich syndrome[10], which, unlike the mouse disease, is accompanied by defects in T-cell and platelet function. Arguing that the mouse *xid* genes affected in X-linked immune deficiencies would be expressed in lymphocytes[7], Cohen *et al.* sought *xid*-related messenger RNA by the 'subtraction' cloning technology developed for isolating the T-cell receptor genes[11,12]. Of the 15 lymphocyte-specific cDNA clones thus prepared, one clone, which is apparently T-cell specific, recognized an X-linked gene family that might contain as many as 15-20 members. These genes have been named *XLR* (X-linked, lymphocyte-regulated)[7].

Two lines of circumstantial evidence suggest that the *XLR* genes are directly related to the *xid* locus. First, genetic studies with congenic strains of mice show there to be very close linkage between the *xid* locus and the *XLR* locus. Second, detailed expression studies detect *XLR*-derived mRNA not only in T cells but also in plasma cells and other B cells at late stages of differentiation. Unlike plasmacytomas derived from normal mice, plasmacytomas from *xid* mutant mice do not contain *XLR* transcripts[8]. Independent evidence has implicated mature B cells as the target tissue for the *xid* locus[9]. So, either the *xid* locus is regulating the *XLR* locus or the two loci are identical. Final proof of identity will require demonstration of an alteration in an expressed *XLR* gene that is the result of the *xid* mutation.

The X-chromosome of eutherian mammals has been conserved during evolution[13] and the presence of an *XLR* locus in humans can be predicted confidently. It will be of obvious interest to study *XLR* expression in Wiskott-Aldrich syndrome and in the other human X-linked, immunodeficiency syndromes. Even if mutation at the *XLR* locus is not directly responsible for disease, this gene family is certain to be studied in detail. With a developmentally-regulated expression that is apparently restricted to lymphocytes, and with the possibility that different members of the gene family are transcribed at different times, the *XLR* genes may well be responsible for regulating lymphocyte development and are therefore bound to be extensively examined in the coming months □

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1. Waldman, T.A., Strober, W. & Blaese, R.M. in *Clinical Immunology* Vol. 1 314 (W.B. Saunders, Philadelphia, 1980).
2. McKusick, V.A. *Mendelian Inheritance in Man* (John Hopkins University Press, 1983).
3. *Human Gene Mapping* 7; *Cytogenet. Cell Genet.* 37, 1 (1984).
4. Collins, M.K., *et al. EMBO J.* 3, 2347 (1984)
5. Collins, M.K. *et al. Nature* (in the press).
6. Stinson, A. *et al.* (submitted).
7. Cohen, D.I. *et al. Nature* 314, 369 (1985).
8. Cohen, D.I., Steinberg, A.D., Paul, W.E. & Davis, M.M. *Nature* 314, 372 (1985).
9. Scher, I. *Adv. Immunol.* 33, 1 (1982).
10. Cooper, M.D., Chae, H.P., Lowman, J.T. Krivit, W. & Good, R.A. *Am. J. Med.* 44, 499 (1968).
11. Hedrick, S.M., Cohen, D.I., Nielsen, E.A. & Davis, M.M. *Nature* 308, 149 (1984).
12. Davis, M.M. *et al. Proc. natn. Acad. Sci. U.S.A.* 81, 2149 (1984).
13. Ohno, S. *Monograph Endocrinol.* 1 (Springer, 1967).

Molecular biology

Coordination of sequence data

from Arthur M. Lesk

MANY challenges are presented by the very rapid growth of DNA and protein sequence information, the opportunities arising from advances in computer data-base management facilities, and the variety and importance of the applications of this knowledge. Not least is the challenge of coordinating the various data-banking projects.

The main archives of nucleic acid sequences, at the European Molecular Biology Laboratory Library and GenBank (a collaborative effort between Los Alamos National Laboratory and Bolt, Beranek and Newman), now contain approximately 3×10^6 bases, about half of the total of published sequences. It is estimated that another 5×10^5 bases are currently being published every six months, and the process is still accelerating.

The major archive of amino-acid sequences of proteins (now often derived from gene sequences) is the Protein Information Resource, based at the National Biomedical Research Foundation in Georgetown, Virginia, now under the direction of W.C. Barker. It contains over 3,000 entries, comprising over 6×10^5 amino-acid residues. R.F. Doolittle of the University of California at San Diego, and S. Sakakibara of the Protein Reseach Foundation, Osaka, Japan, also maintain protein-sequence data banks. In addition, numerous specialized collections exist, such as the 'Sequences of Proteins of Immunological Interest', published by the US National Institutes of Health, and the International Haemoglobin Information Center, which is directed by R.N. Wrightstone of the Medical College of Georgia.

The Protein Structure Data Bank at Brookhaven National Laboratory, directed by T.F. Koetzle, contains, in its latest release, 253 sets of atomic coordinates of proteins and nucleic acids, almost all derived from X-ray crystallography.

Current activities fall into four categories. First, there is data acquisition by an archive. The basic chain of events at present is: publication; surveillance of the literature and extraction of published data by the archiving project; quality control; editing, formating and addition to data base. Second, the archived information is distributed on magnetic tape or, if only small amounts of information are involved, over computer networks. Third, there is information retrieval, dependent on the development of computer program systems as interfaces between archived information and research projects. Finally, there are applications — for example, the search of a data base for sequences similar to the one that has been newly determined.

Those of us whose activities primarily involve applications depend, ever more crucially, on the quality, completeness and accessibility of the archives. Despite the stake of the scientific community at large in these resources, individual projects have so far borne the responsibility for collecting, maintaining and distributing the data. In contrast, many groups all over the world are developing software for information retrieval and research applications in molecular biology. To the extent that the computer programs are well described and readily distributed, they are subject to ordinary processes of natural selection from which, one may presume, the most effective will ultimately emerge in common use.

How can new generations of molecular biologists and new generations of com-

puters interact most effectively? Certain algorithms and computer programs are already mature and applicable directly to the data bases that are increasing so fast in scope. The introduction of very large, fast computers and the design of special-purpose chips will have several effects. Certain tasks, for which computational power is the limiting resource, will for the first time become feasible. The prediction of protein structures from sequences may fall into this category, although there is considerable pessimism about the adequacy of the models on which such calculations have so far been based. Faster computers will also enable some tasks, now possible but slow, to be performed interactively. This will stimulate changes in the design of algorithms and user interfaces.

The intimate combination of graphical and numerical approaches to computational molecular biology will continue to be essential. A scientist analysing a protein or nucleic acid structure will want to ask a variety of questions. The computer's response may take the form of a picture, or text, or both. When possible, the program will respond at conversational speed, to establish a dialogue between user and program. Which tasks are interactive and which must be 'spun off' into batch queues must depend on the hardware configuration.

Until now, many programs have been designed to answer descriptive questions about protein sequences or structures. To design systems that address more complicated questions, such as "What if...?" or "What is the relationship between...?", the user must be able to perform computational experiments that modify the structures. Such questions will require more powerful interactive and manipulative tools, and are likely to involve the development of expert systems.

A major problem in creating programs for a worldwide community of users is the design of supple software, adaptable to the needs of different users. A very general concept suggested some time ago may provide a useful approach. This is the 'virtual data base' — an information-access system that is self-modifiable, under user control. Each session of each user will produce an 'active state' in which certain working files of structures have been created, identified and linked, and certain groupings of elementary operations defined to create a working set of higher-level commands and procedures. It will be useful to be able to save, restore and manipulate the 'active state of the data base', or to define a 'start-up procedure' to establish, at the beginning of any session, a state tailored to the individual investigator or even to the particular project.

The need to establish a mechanism for coordination stems not from any dissatisfaction with the operation of information management projects currently in existence, but from the fear that Malthusian growth will saturate the channels of data acquisition, distribution, and utilization. To meet the need, CODATA (the Committee on Data of the International Congress of Scientific Unions) has established a Task Group on coordination among data banks. This group, chaired by Professor B. Keil of the Institut Pasteur, and comprising representatives from Europe, North America and Japan, has been charged with enhancing international cooperation in archiving, distribution and utilization of information related to protein sequences.

General problems that the CODATA task group plan to address include:

(1) Maintaining directories of protein sequence data bases* — including the special features of their contents or support facilities, addresses of their directors, and availability — and of computer programs for data retrieval and applications.

(2) Encouraging the establishment of guidelines and use of standardized nomenclature, and the development and general acceptance of common formats for data exchange.

(3) Establishing links between protein sequence data banks and data banks containing related information, such as nucleic acid sequences or protein structures.

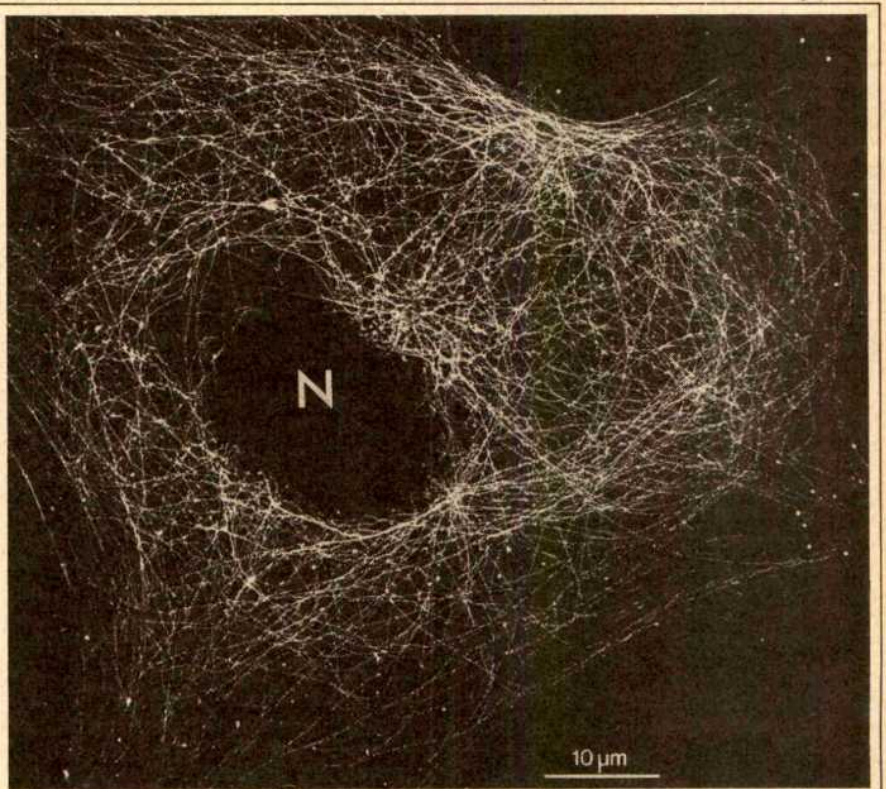
(4) Fostering coordination of efforts to avoid duplication and to maximize the effectiveness of the work of different groups in different countries; for example, by identifying gaps in current programs and encouraging the development of specialized data bases when appropriate.

(5) Guiding the design of distributed material to enhance the general utility of the data banks, taking into account the needs of scientists without sophisticated familiarity with computer programming or those with access to limited computer resources. This may include the development of introductory tutorial documents for new users, or sponsoring training courses as satellite sessions of scientific meetings.

The effective coordination of the efforts of one scientist and one computer working together on a single project is primarily a technical one. The effective coordination of the efforts of many scientists, working separately on different projects, requires the personal touch as well. It is hoped that the CODATA Task Group will be able to pay due attention to both sorts of problems, and thus be able to channel the force of the information explosion in molecular biology into even more productive directions. □

*The committee would be grateful if individuals who have assembled such data banks would write to the chairman, so that the catalogue may be as complete as possible.

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Microtubule network of an epithelial cell visualized by immunophotoelectron microscopy. N is the nucleus. The technique combines photoelectron imaging with immunofluorescence. For this example, the cell was first treated with a monoclonal antibody to microtubules, then with a fluorescently-labelled antibody to the first antibody, and finally with a colloidal gold-labelled antibody to the second antibody. The second stage was used to enable the technique to be compared with immunofluorescence microscopy, which has a resolution of 200 nm, whereas the resolution of immunophotoelectron microscopy is 10-20 nm. From Birrell, G.B., Hablston, B.L., Nadakavularen, K.K. & Griffith, O.H. Proc. natn. Acad. Sci. U.S.A. 82, 109; 1985).

Astronomy

Supernovae and the distance scale

from Sidney van den Bergh

IN SPITE of fifty years of observational effort, the Hubble constant, which measures the size (and somewhat less directly the age) of the Universe, remains poorly determined. That is so because galaxies are extremely distant and so the 'standard candles' within them are exceedingly faint. The most luminous known standard candles are supernovae of type I (SNI) at maximum light. On page 337 of this issue, W.D. Arnett, D. Branch and J.C. Wheeler suggest that it may be possible to calibrate the maximum luminosity of SNI directly from nuclear physics. This approach bypasses the traditional and error-prone 'distance ladder' technique for the determination of the extra-galactic distance scale.

It is now generally believed that the precursors of SNI are mass-accreting carbon/oxygen white dwarfs in close binary systems. The C-O nuclear fuel in these objects will ignite if mass transfer from a binary companion pushes the total mass of the white dwarf above about 1.4 solar masses (the Chandrasekhar limit). Nuclear incineration of C-O to radioactive ^{56}Ni in an exploding white dwarf will produce 1.4×10^{51} erg per solar mass. Most of this energy ends up as kinetic energy of matter ejected during the supernova explosion.

The optical luminosity of SNI is believed to result from the trapping and thermalization of the γ rays and positrons emitted by the decay of ^{56}Ni through ^{56}Co to ^{56}Fe. Detailed hydrodynamical calculations have indicated that a C-O white dwarf that is pushed over the Chandrasekhar limit will produce 0.4 - 1.4 solar masses of ^{56}Ni. Based on these calculations and other evidence, the best estimate of Arnett *et al.* is that a SNI will produce 0.6 solar masses of ^{56}Ni and that the decay of one solar mass of ^{56}Ni would produce a maximum supernova luminosity of $(2.2 \pm 0.2) \times 10^{43}$ erg s^{-1}. They then deduce, from knowledge of supernova spectra, that the decay of 0.6 solar masses of ^{56}Ni will produce a supernova with a maximum blue magnitude, M_B(max), of -19.5, with limits of -20.4 and -19.1, corresponding to 1.4 and 0.4 solar masses of ^{56}Ni.

For SNI that have been observed to occur in elliptical and lenticular galaxies (which are generally dust-free) with redshifts larger than 3,000 km s^{-1} (for which random motions of individual galaxies can be neglected), Arnett *et al.* find that $M_B(\max) = -18.4 + 5 \log (H/100)$. Substitution of their calculated value of M_B(max) into this relation yields $H = 59$ km s^{-1} Mpc^{-1}, with upper and lower limits of 73 and 39 km s^{-1} Mpc^{-1}.

This result places Arnett *et al.* firmly on the side of those who find that the Hubble constant is small enough to accommodate the age of the Universe derived from evolutionary age models of globular cluster stars — about 1.8×10^{10} years. Those who favour larger values of the Hubble constant and smaller ages of the Universe will, no doubt, bring forward four arguments. First, incineration of a carbon/oxygen white dwarf might, in fact, produce less than 0.4 solar masses of nickel. Second, calculation of the maximum optical luminosity of SNI involves rather complex radiative transfer calculations in a supernova shell in which physical conditions are not yet perfectly understood. Third, more photoelectric observations of supernovae are required to support the assumption that all SNI have the same luminosity at maximum light. And, fourth, it is not yet entirely certain that SNI at maximum light are well represented by spherical black bodies with a temperature of 20,000 K.

As long as such uncertainties remain, the search will continue for additional calibration techniques for the extra-galactic distance scale. New urgency has been given to this search by the recent work of A.R. Walker (*Mon. Not. R. astr. Soc.* **212**, 343; 1985) which suggests that the calibration of the Cepheid distance scale may be in error by as much as 0.2 - 0.3 mag. Promising new methods for the calibration of the extra-galactic distance scale, which have not yet been fully exploited, involve observation of the light curves of distant novae (two of which have recently been discovered in the Virgo cluster galaxy M87) and comparison of the luminosity functions of distant globular systems with those of M31 and the Galaxy. Such determinations are now possible by detecting the maxima of the luminosity functions for the distant globular cluster systems surrounding NGC3379 and M87. The ultimate solution to the distance-scale problem may have to await the Space Telescope. □

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Palaeontology

Scottish window on terrestrial life in the Lower Carboniferous

from Andrew R. Milner

ONE of the areas of the world that has the greatest potential for filling some of the gaps in our knowledge of the land fauna before the middle stage of the Upper Carboniferous — the Westphalian (316–296 Myr) — is the Scottish Midland Valley around and between Edinburgh and Glasgow. This area is covered by sequences of freshwater, estuarine and marine beds and there are many coal-mines and surface workings cutting through the appropriate horizons. Over the last decade, the palaeontological community has benefited greatly from sustained prospecting in that area by Mr Stanley Wood. Amongst other new sites, he has revealed an early Upper Carboniferous lake-bed assemblage of aquatic amphibians and fishes at Cowdenbeath[1,2]; an early Upper Carboniferous coastal assemblage of fishes and crustaceans at Bearsden[3]; and, as reported on page 355 of this issue, the first Lower Carboniferous assemblage of terrestrial amphibians, arthropods and plants at East Kirkton[4].

The origin and early diversification of terrestrial animal life is a vast palaeontological and evolutionary jigsaw of which we still have only a handful of pieces. The first certain terrestrial arthropods — from the Rhynie Chert and the Old Red Sandstone — are of Lower Devonian age (410 - 400 Myr), whilst the earliest known amphibians are Upper Devonian (around 360 Myr) and already show few relictual fish-like characteristics. To cover the intervening time span, some half-dozen localities have produced only fragments of information about terrestrial arthropods, although the recently reported Middle Devonian locality at Gilboa, New York State[5] shows great promise. The record has until now been little better for the early Carboniferous and it is not until the Westphalian that palaeobiologists can study comprehensive assemblages of myriapods, arachnids, insects, molluscs and vertebrates. Many groups of terrestrial animals make their first appearance as fossils in the Upper Carboniferous, in particular the winged insects and reptiles, but their very structural diversity at this time speaks for a long previous history. By the time we can see what is happening on land, many of the most interesting events have already taken place.

This, then is the significance of the East Kirkton assemblage, which is quite unlike any previously described association of Lower Carboniferous/Mississippian animals. It seems to be in a spherulite-bearing freshwater limestone laid down in shallow pools associated with hot springs[6]. No fish or benthic invertebrates are present, suggesting that the pools were generally uninhabitable. To my knowledge, only two Palaeozoic sites, both younger, show environmental similarity

to that at East Kirkton. One is an algal limestone from Hamilton in Kansas, which appears to be a very similar matrix but without the spherulites, and is of late Pennsylvanian or early Permian age. It has produced a similar association of temnospondyls, arthropods and plants[7] but differs in the presence of at least one genus of fish being present in abundance[8]. A Lower Permian freshwater limestone of rather different character from Niederhässlich, near Dresden, also has a large fauna of small amphibians and reptiles without fish[9]. A comparative study of these sites might ultimately permit us to identify common geological factors and so to predict further such localities.

The predominant amphibian in the East Kirkton assemblage bears a close resemblance to *Dendrerpeton*, a temnospondyl amphibian from the early Westphalian of Nova Scotia and Ireland[10,11]. The Nova Scotia material was found in accumulations inside hollow lycopod stumps, suggesting preservation under terrestrial conditions[12], and it is generally believed that *Dendrerpeton* was a terrestrial animal. The East Kirkton amphibian is the earliest certain temnospondyl, showing the definitive large tympanic notch and the huge openings in the palate that characterize the amphibian order Temnospondyli and its descendants — the frogs.

The few months of collecting at East Kirkton have produced intact amphibians, millipedes, eurypterids, scorpions, the earliest harvestman and much plant material, some of it three-dimensionally preserved in chert. If collecting can be extended, the material should establish whether the Euramerican Lower Carboniferous continental fauna was significantly more primitive than its Upper Carboniferous equivalent. If they were similar, the problems of early terrestrial evolution and diversification are pushed back into the Devonian.

As one of the earliest sites to produce most of the components of a terrestrial ecosystem, East Kirkton deserves comprehensive investigation. But there are difficulties in attracting funds for such projects, which makes it increasingly necessary for palaeontologists to adopt a higher public profile. Following the successful dinosaur exhibition put on by York Museum with the cooperation of the British Museum (Natural History), the Hunterian Museum of the University of Glasgow is preparing an exhibition based on Stanley Wood's remarkable collections, to include some of the East Kirkton material and the spectacular Bearsden fishes. It will travel around museums in Scotland and visit the British Museum (Natural History) in London during 1986. □



Scorpion (×4) from East Kirkton.

1. Andrews, S.M. *et al. Nature* **265**, 529 (1977).
2. Smithson, T.R. *Palaeontology* **23**, 915 (1980).
3. Wood, S.P. *Nature* **297**, 574 (1982).
4. Wood, S.P. Panchen, A.L. & Smithson, T.R. *Nature* **314**, 355 (1985).
5. Shear, W.A. *et al. Science* **224**, 492 (1984).
6. Muir, R.O. & Walton, E.K. *Trans. Geol. Soc. Glasg.* **22**, 157 (1957).
7. Mapes, G. & Rothwell, G.W. *Palaeontology* **27**, 69 (1984).
8. Zidek, J. *Paleont. Contr. Univ. Kansas* **83**, 1 (1976).
9. Credner, H. *Naturwiss, Woch.* **5**, 471 (1890).
10. Carroll, R.L. *J. Paleont.* **41**, 111 (1967).
11. Milner, A.R. *Palaeontology* **23**, 125 (1980).
12. Carroll, R.L. *et al. Field Excursion A59 Guidebook, 24th Int. Geol. Congr., Montreal* (1972).

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Cytoskeleton

Myosin filaments in cytoplasm

from Thomas D. Pollard

EVERY so often a quiet and contented field of research is awakened by a technical advance that at once changes the conventional wisdom and opens up new avenues for experiments. The paper by Yumura and Fukui in the 14 March issue of Nature[1] should have this impact on investigation of myosin filaments in non-muscle cells.

Myosin is best known as a filamentous protein of muscle, in which, together with actin, it plays a major role in the process of contraction by the sliding-filament mechanism. Much less is known of the function of myosin in non-muscle cells and its presence in filamentous form has been in doubt. Yumura and Fukui have shown for the first time, in either the light or electron microscope, both the extent of polymerization of myosin and the distribution of its filaments inside a non-muscle cell. Yumura and Fukui devised a simple method to visualize the filaments. The trick involves flattening live cells with an agar overlay[1] to create a more or less two-dimensional cell, in which fine details are not obscured by superimposition, and individual myosin filaments, stained with a fluorescent antibody, can be resolved in the light microscope[2]. Although only demonstrated for the cellular slime mould *Dictyostelium discoideum*, the technique should be widely applicable.

Previous work with fluorescent antibody staining of vertebrate cells in culture has established that myosin is associated with actin-containing stress fibres[3,4] and the cleavage furrow during cell division[4]. The staining was often reported to be punctate, but it has not been possible to resolve individual filaments. It has also been difficult convincingly to demonstrate myosin filaments in non-muscle cells by electron microscopy, even using antibodies[5-7]. The general view has been that myosin filaments are present, but too small to visualize by light microscopy and so few in number that they are lost to view in electron micrographs among the abundant actin filaments.

The new technique makes it possible to identify individual rod-shaped particles in cells that are stained with a fluorescent antibody to myosin. Although there is no direct proof that these particles are myosin filaments, it is very likely so, because they are the same size and shape as the filaments prepared from purified myosin.

Yumura and Fukui are able to make several important points on the basis of their observations. First, the bulk of the myosin is polymerized into filaments in the cell. Second, they confirm that myosin is concentrated in the cleavage furrow of dividing cells but add that the filaments are oriented parallel to the band of actin filaments that encircles the equator of the cell. This is the alignment expected if the myosin interacts with actin filaments in a sliding-filament mechanism to produce the force required for division. This observation strengthens arguments for such a model[4,9,10]. Third, they show that cyclic AMP, the chemoattractant for *Dictyostelium*, causes a striking transient redistribution of the myosin filaments in the cell. This change may very well be a primary step in the chemotactic response of the cell to cyclic AMP and we can look forward to learning whether it is caused by phosphorylation of the myosin[11] or some other mechanism. Finally, evidence is provided that the myosin filaments are destroyed by treatment with osmium. If this is true for myosin in other cells, it may well explain previous difficulties in demonstrating myosin filaments by electron microscopy □

1. Yumura, S. & Fukui, Y. *Nature* **314**, 194 (1985).
2. Yumura, S., Mori, H. & Fukui, Y. *J. Cell Biol.* **99**, 894 (1984).
3. Weber, K. & Groeschel-Stewart, U. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4561 (1974).
4. Fujiwara, K. & Pollard, T.D. *J. Cell Biol.* **71**, 848 (1976).
5. Herman, I.M. & Pollard, T.D. *J. Cell Biol.* **88**, 346 (1981).
6. Drenckhahn, D. & Groeschel-Stewart, U. *J. Cell Biol.* **86**, 475 (1980).
7. Hirokawa, N., Tilney, L.G., Fujiwara, K. & Heuser, J.E. *J. Cell Biol.* **94**, 425 (1982).
8. Clarke, M. & Spudich, J.A. *J. molec. Biol.* **86**, 209 (1974).
9. Schroeder, T.E. *J. Cell Biol.* **53**, 419 (1972).
10. Mabuchi, I. & Okuno, M. *J. Cell Biol.* **74**, 251 (1977).
11. Kuczmarski, E.D. & Spudich, J.A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7292 (1980).

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Plasma physics

Fusion in magnetized plasmas

from A.C. Riviere

FUSION of light nuclei under controlled conditions promises to be a source of energy for electricity generation in the future. The latest acheivements in plasma physics and controlled nuclear fusion, many of which were reported at a recent conference*, make it clear that considerable progress has been made towards the conditions needed for 'ignition', when energy lost from the plasma is matched by energy gained from alpha particles produced by fusion reactions. In a plasma that is half deuterium and half tritium, this requires the product of nuclear density (N) and energy confinement time τ_E to exceed about 2×10^{20} m^{-3}s with the temperature above 10 KeV (10^8 K).

The main approach to fusion has been to use a magnetic field to isolate the hot plasma from the vacuum vessel wall, and the most successful magnetic field geometry is that of the 'tokamak' which combines a strong toroidal field with the field due to a toroidal current flowing in the plasma. A record value of 0.8×10^{20} m^{-3}s for $N\tau_E$ at 1.5 KeV has been achieved in the Alcator C tokamak at MIT (M. Greenwald) by injecting solid hydrogen pellets into the ohmically heated plasma. This method of refuelling raises N without causing a reduction in τ_E. In a much larger tokamak, Doublet III at San Diego, a value of 0.14×10^{20} m^{-3}s for $N\tau_E$ at 6 KeV has been achieved by combined neutral-beam heating and hydrogen-pellet refuelling in a divertor configuration. In the latter variant of the tokamak, magnetic field lines are arranged to connect the plasma edge to a remote collector rather than have the plasma edge defined by a local solid surface. This allows some control over the flow of impurity ions into the plasma and thereby a reduction in radiation losses. Experiments at ASDEX, Max Planck Institut, Garching and PDX, Princeton, also show improved performance with a divertor, particularly in allowing the maintenance of a second plasma state, called the H-mode, the τ_E of which is about a factor of 2 better than that of the more usual state. In the H-mode a steep rise in temperature occurs at the plasma edge but whether the divertor configuration is essential for this to be formed is not yet clear.

Although plasma densities and temperatures close to those required for ignition have been achieved separately at various times, it remains difficult to reach the necessary energy confinement time. Energy is lost from the plasmas in fusion experiments at an anomalously high rate compared with that expected from the effect of electron-ion collisions. Until recently the scaling of τ_E with size for tokamaks was though to vary as the square of the scale size — in particular, $\tau_E \propto a^2$, where a is the minor radius of the plasma — so larger and larger machines have been built. With results now available from a wide range of machines it is, instead, found that τ_E increases as the cube of the scale size, probably as $\tau_E \propto aR^2$, where R is the major radius of the torus (P.C. Efthimion, TFTR, Princeton). The Joint European Torus (JET), the largest tokamak built so far with $R = 2.96$ m and $a = 1.25$ m, started operating about a year ago and its performance is at least as good as was originally hoped — with τ_E about 0.8 s — and confirms the scaling with aR^2 (P. Rebut and J.G. Cordey). Plasma currents approaching 4 MA have been achieved in JET and 25 MW of additional plasma heating power is soon to be installed. This is expected to drive the plasma density and temperature up to the conditions where the power deposited by the α-particles from thermonuclear reactions will play a major role (P. Rebut).

Many new experiments have been successful in improving energy confinement. For example, injection of deuterium instead of hydrogen beams into deuterium plasmas at Doublet III and ASDEX, refuelling with pellets rather than feeding gas at the plasma boundary at Alcator C and Doublet III, addition of low Z impurity ions at ISX-B, Oak Ridge, and the use of magnetic divertors at ASDEX, Doublet III and PDX. An explanation for all these effects did not emerge at the meeting, although careful construction of the relative shape of temperature and density profiles may be one factor in minimizing the cross-field energy flow to the walls.

A clear answer is, however, emerging on the maximum value of the ratio (β) of plasma to magnetic-field pressure that can be supported by tokamaks with present techniques. Many experiments have found a β limit, independent of the form of heating, given by $\beta = \text{const} \times I/(aB)\%$, where I is the plasma current and B the value of the toroidal field at the plasma centre. The maximum value of the constant achieved in experiments at Doublet III, ASDEX, PDX, and both CLEO and TOSCA (Culham), is about 3.5, which is in quite good agreement with theoretical calculations by several groups. Beyond this value of β, the τ_E decreases rapidly or the plasma completely breaks up ('disrupts') and the plasma energy is lost. The currently observed limit to β is rather restrictive for present thermonuclear reactor designs based on the tokamak, because their values of I/aB are too small. Some re-thinking of typical reactor parameters is now needed. It should be recalled that there is a limit (the Kruskal/Shafranov limit) above which the plasma current of a tokamak cannot be increased; at higher currents the plasma usually disrupts and terminates the discharge.

The ability to contain a higher value of β is expected for the reversed field pinch (RFP) — a toroidal magnetic system in which the toroidal field is reduced relative to that of a tokamak and is comparable with the poloidal field. An RFP achieves gross stability by reversing the toroidal field in the outer plasma layers, thereby providing a region where the field-line pitch changes rapidly with radius (high shear). Although the development of RFPs is somewhat behind that of the tokamak, D.A. Baker (Los Alamos National Laboratory) reported that an electron temperature of 0.5 KeV has been achieved in the ZT-40M experiment with a plasma current of 0.4 MA; the discharge duration had been improved from a few ms to 27 ms at lower currents. Similar progress was described by P.G. Carolan (Culham) from the performance of the HBTX1A experiment. These parameters move the RFP performance towards that of tokamaks of the

*10th international conference on 'Plasma Physics and Controlled Nuclear Fusion', Imperial College, London 12-19 September 1984.



100 years ago

THE PEABODY MUSEUM AT NEW HAVEN, U.S.

THE Peabody Museum, Mr. Ingersoll informs us, stands on the corner of Elm and High Street, just without the *campus* of Yale College. The building is due to the liberality of George Peabody, who gave a sum of money, in 1866, to erect a house for the collections. Thanks to the financial prosperity of Massachusetts, the bonds for a hundred and fifty thousand dollars had greatly increased, and those set aside for the first wing of the building had become worth a hundred and seventy-five thousands dollars when the trustees began to build. With that sum they have erected one of the finest buildings, for its purpose, in the United States—a lofty and ornamental structure of red brick and cream-coloured stone, whose broad and numerous windows expresss the desire of the investigators within for all the light they can get.

The glory of the Yale Museum is its palæontological treasures, brought together wholly by Prof. O. C. Marsh. The few representatives of this collection visible in the second-floor and in the hall-ways are alone sufficient to stamp the museum as pre-eminent of this line; but they are merely an advertisement of what cellar and attic contain. It is not too much to say that, in respect to vertebrate palæontology (outside of fishes), this museum is not surpassed in the world. Where other collections own fragments or single skeletons, Prof. Marsh boasts scores or hundreds of individuals.

From *Nature* **31** 510, 2 April 1885.

same size and, according to one theoretical model, the performance of an RFP at the reactor level could exceed that of the tokamak (J.B. Taylor, Culham).

Another approach to maintaining a stable discharge at high values of plasma current is to apply a helical field of the correct helicity from outside. OHTE was built to test this hypothesis and T. Tamano (San Diego) reported that a plasma current of 0.5 MA had been maintained in OHTE for several milliseconds, giving electron temperatures of 0.5 KeV, although the desired field configuration may not have been completely established. The β values achieved by the RFPs and OHTE are around five per cent and so comparable with those from well-maintained tokamaks, but are obtained at the cost of very high power input, indicating a very short τ_E. Thus, to make their use in power stations more economically attractive, tokamaks will need higher β values, whereas the RFP and OHTE devices will need to achieve very much larger values of τ_E.

The spheromak type of configuration has affinity with the RFP but the plasma is formed as a free toroid that generates its own field so that a central conductor through the hole in the plasma doughnut is not required. M. Yamada (Princeton) reported successful generation and control of such plasmas in the S1 spheromak for a period of one millisecond at a plasma current of 0.35 MA, but with an electron temperature of only ~ 50 eV. The CTX (Los Alamos) and CCTC-I (Osaka) experiments have also generated such plasmas. Although at an early stage of development, these intriguing devices should tell us a lot about the ability of magnetized plasmas to regulate themselves by automatically relaxing and maintaining a preferred magnetic geometry.

Stellarators are an important class of field configurations which require no plasma current and entirely confine the plasma by the use of externally-generated helical fields. Heliotron E is stellarator with a highly-sheared magnetic field. It can achieve a β value of 2 per cent (K.Uo, Kyoto) and more power may take the value yet higher. Theoretical limits to β in stellarators, discussed by B.A. Carreras (Oak Ridge) and J. Johnson (Princeton), are comparable to those for the tokamak. Wendelstein VII A (Garching) is a weak shear $\lambda = 2$ stellarator in which, like Heliotron E, the plasma is heated both with neutral beams and with absorption of microwaves at the electron cyclotron resonance. In this machine, τ_E shows a strong sensitivity to the rotational transform with large dips in τ_E at rational values (H. Renner, Garching). The weak shear in this case probably allows large regions of the plasma to be interconnected because of the effect of small resonant perturbations. Overall, however, stellarators are now improving on the performance of tokamaks of comparable size, and producing τ_E values closer to the classical values expected due to collisions alone. Their value of τ_E can therefore be expected to increase as B^2, a feature not observed in tokamaks; this should be confirmed in the new devices now being constructed at Garching (WVIIAS) and at Oak Ridge (ATF). As stellarators do not require a plasma current they can be operated continuously without plasma disruption. The complex coil system required to establish the helical field is a serious drawback to stellarators but simpler coil designs, which would avoid most of the engineering problems, have been proposed.

The mirror machines, with their essentially linear geometry, are the most studied non-toroidal systems. Many of these devices, some quite large, are in operation. Very ingenious techniques are used to prevent electrons, ions and energy being lost too rapidly along the field lines. In TMX-U (Livermore) and Gamma 10 (Tsukuba), the formation of potential barriers and improved energy confinement have been demonstrated. However the plasma parameters in the centre ($N = 6 \times 10^{18}$ m^{-3}, $T = 0.2$ KeV and $\tau_E = 5$ ms) are still modest compared with toroidal systems.

The heating of plasmas above that provided by the plasma current is technically difficult and has, until recently, relied on injection of fast particle beams. Heating by the absorption of radio-frequency waves has now advanced to the point where MWs of power can be delivered to the plasma. In particular, at the electron cyclotron resonance, radiation with wavelengths of 3.6 and 5 mm and a power level of 1 MW has been used very effectively both in the Doublet III tokamak (K.H. Burrell) and in the T10 tokamak in Moscow (V.V. Alikaev). The local nature of the power deposition with this form of heating has been used to study energy transport and instability control.

There is no doubt that exciting progress in fusion research has been made in many directions, although extensive development work will be needed for economic power generation from fusion. The next significant step in fusion research is going to be the achievement of ignition and many now believe this can be done. □

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New constraint on biomedical research?

THE recent prosecution of the Royal College of Surgeons, Parliamentary moves to replace the Cruelty to Animals Act (1876) and the search for *in vitro* alternatives to animal experimentation may have far-reaching effects on research in Britain. A further step in this often acrimonious debate was taken at the inaugural meeting of a new pressure group in Oxford last week.

The conference was opened by the ecologist, Dr J.H.C. Finch. He pointed out that we are, of neccessity, closely linked with all the living organisms with which we share the planet, and that we ignore this at our peril. The wholesale destruction of ecosystems, nuclear proliferation and unethical experimentation were all part of the same anti-life attitude.

Citing electrophysiological evidence that even plants, although they lack a demonstrable nervous system, are capable of responding to painful stimuli, Dr Finch said, "We should respect and revere all the organic world". In the following discussion, several participants pointed out that large and effective organizations already exist for animal rights and suggested the formation of a parallel group to protect plant life. One delegate suggested the term 'chlorophyll based organisms' or CBOs, because they felt the word 'vegetable' can carry derogatory connotations. However, after debate, the name Vegetable Liberation Front was accepted for the new organization.

On the question of agriculture, it was agreed to demand that the government support a major research initiative to develop methods for the *de novo* molecular synthesis of foodstuffs. Until this was achieved, the spraying of crops with local anaesthetic before to reaping was recommended. No agreement was reached on the ethics of genetic engineering of bacteria for mass production of nutritional protein. One faction insisted that transfection is a violation of genetic integrity, and that this constitutes a subtle form of oppression. "Even bacteria have rights", said one speaker.

There was a strong feeling expressed by many that the movement could only develop, like the Animal Liberation Front, through direct action. Tactics for harvest sabotage, ranging from the immobilization of tractors (but not by stuffing potatoes in their exhaust pipes) to the occupation of grain silos were discussed. Attacks on herbicide factories were rejected because "they could serve a useful role in culling the human predator." The international nature of the struggle was stressed by a fraternal delegate from Holland. He proposed simultaneous demonstrations throughout the EEC outside offices of Interflora, a multinational corporation accused of cruelty to flowers.

Given the urgency of the problems raised, the question of a constitution was deferred until a subsequent meeting. However, a manifesto was drawn up and agreed by the overwhelming majority of delegates. The document was engraved on tablets of stone, since the manufacture of paper was held to entail the suffering of trees, and a copy was posted through windows of the editorial offices of this and other journals.

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Amazonian Indians and the forest environment

SIR — The statement in a recent *News and Views* article by May[1] that Amazonian Indians possess a much greater knowledge of their environment than has been commonly believed prompts me to communicate some data gathered recently that further support this contention.

The Institute of Economic Botany of The New York Botanical Garden (supported by E. J. Noble Foundation) is engaged in a four-year comparative ethnobotanical study on the question "How important is the forest to Amazonian Indians?" Much has been written on the significance of the forest to Amazonian Indians[2], but rarely has the degree of importance been quantified, and then only on a very small scale[3]. A primary goal of the project is to quantify forest utilization for 1 hectare of forest with each tribe studied.

The Chacobo Indians of Bolivia were the first to be studied[4]. Just 30 years ago, the tribe numbered only about 135 individuals who essentially lived outside the Bolivian cash economy by hunting, fishing, and subsistence agriculture in isolated forest locations along the Ríos Ivon, Benicito, Geneshuaya and Yata in the northeastern Bolivian department of Beni[5-7]. In 1955, modern missionary work with the Chacobo began and in the ensuing years a number of aspects of their culture, including their ceremonies, traditional mode of dress, and supernatural beliefs have been lost. Other aspects, including their botanical knowledge, are to a greater degree intact, at least among the elders. Although their numbers have increased to about 400, their culture is on the verge of extinction. The majority of the tribe (some 280 individuals) live today on 43,000 hectares of forested land along the Río Ivon, and on this reserve make their capital village, Alto Ivon (11°45'S, 66°02'W). The vegetation around Alto Ivon is Tropical Moist, terra firme forest. The elevation is about 200 m and the topography is level. The inventory, which was carried out in January and February 1984, was made on a strip of undisturbed forest 10 × 1,000 m. Every tree with a DBH (diameter at breast height) of 10 cm or more was marked and collected. Indigenous names and use information were obtained from Chacoba informants.

The inventory showed that 91 species, represented by 649 individual trees, occurred in the hectare. Of these, the Chacobo had uses for 75 species (82% of the total), or 619 individual trees (95% of the total). The species were grouped into five classes of utilization: food, fuel, crafts and construction, medicinal, and commercial. Some species, especially the palms, had uses in more than one category. There was only one commercially exploited species, wild rubber (*Hevea brasiliensis*), and 5 individuals of that species were found. Fourteen species (163 individuals) were used specifically as a fuel for cooking, smoking rubber, or both. Thirty-three species of trees (264 individuals) provided edible wild fruits. Twenty-three species (225 individuals) were collected that were used in crafts and construction. Finally, 23 species of trees (271 individuals) were found in the sample hectare that were used by the Chacobo for medicinal purposes.

The demonstration that the Chacoba utilize 82% of the tree species in their environment has significant implications not only for the thesis that such tribes have a profound knowlege of their forest, but also for those individuals concerned with the protection of indigenous cultures in Amazonia. For example, by combining this sort of ethnoecological information with the data being obtained from the Minimal Critical Size of Ecosystems Project (cosponsored by the Brazilian National Institute for Amazon Research and the World Wildlife Fund-US)[8], it should be possible to make more intelligent recommendations as to how much forested land must be allocated for self-sustaining Indian reserves.

Every ethnobotanical study done in Amazonia reaffirms the view that indigenous peoples are scientifically interesting, legitimate and invaluable sources of knowledge about the forest. Since one tribe differs from another in important ways, and since these people are so rapidly losing their traditional knowledge, many more studies are urgently needed. It is clear that botanical inventory involving indigenous cultures, especially in the tropics, can quite justifiably be considered the most worthwhile expenditure of time and effort for this generation of field botanists.

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1 May, R.M *Nature* **312**, 19-20 (1984).
2 Lévi-Strauss, C in *Handbook of South American Indians Vol 3*, (ed J H Steward) 465-486 (Smithsonian, Washington, 1950).
3. Carneiro, R.L in *The Nature and Status of Ethnobotany* (ed R.I. Ford) 201-216 (Univ of Michigan, Ann Arbor, 1978)
4 Webster, B *New York Times*, Sunday, November 4, 1984.
5 Métraux, A. in *Handbook of South American Indians Vol 3*, (ed. J H. Steward) 449-452 (Smithsonian, Washington, 1948).
6 Prost, M D. *Costumbres, Habilidades y Cuadro de la Vida Humana Entre los Chacobos* (Instituto Linguístico de Verano, Riberalta, Bolivia, 1970).
7. Torrico, P B *Indígenas en el Corazón de America* (Los Amigos del Libro, La Paz, Bolivia, 1971)
8 Lewin, R. *Science* **225**, 611-612 (1984)

Electrical aspects of the snowflake crystal

SIR — The discussion "No pattern yet for snowflakes"[1] laments the inability of Vicsek's calculations[2] to reproduce the long-range symmetry observed in snowflakes. In the closing lines it is suggested that adding lattice vibrations to the calculations may be helpful. Because snowflakes are polycrystalline conglomerates there would seem to be little chance that these very short wavelength vibrations could be the mediating phenomenon for symmetry patterns that are many millimetres in size. Long wavelength vibrations have been proposed by McLachlan[3] and later by Tolansky[4]. An inherent drawback in any vibrational theory is that the details of the observed structure must be matched by a spectrum of vibrations with equivalent complexity in amplitude, frequency, and phase. In addition, a mechanism for the generation, transmission, and maintenance of these vibrations must be found.

A simpler, more direct mechanism is desirable to explain the generation of the observed cooperative behaviour in dendrite growth. I have proposed a mechanism that will provide the long-range symmetry observed based on phenomena known to be present during the snowflake development[5]. The fact that snowflakes carry an electric charge has been observed by many workers in the field. The influence of electrical fields on ice crystal growth has been observed by Zawidzki and Papée[6], as well as by Muchnik and Rud'ko in the Soviet Union. The mechanism of symmetry induction is the accretion of water vapour at those sites on the crystall that lead to the lowest potential-energy state. There are twelve symmetry axes to the common snowflake. The axis of each dendrite and the bisector of the angle between dendrites serve as reflection planes to sequentially propagate the pattern to all dendrites. Pohl has described the behaviour of dielectric materials in divergent fields and its commerical application[7].

It is interesting to note that this symmetry-inducing mechanism operates regardless of the sign of the charge of the snowflake, which may change during the growth period, or whether the snowflake is increasing or diminishing in size. It is also independent of the number of dendrites, or their length or configuration.

An examination of a collection of photographs of snowflakes, such as that of Nakaya[8], leaves one impressed by the varieties of type and detail of the symmetry achieved in snowflakes.

It would be most interesting if future calculational models of snowflake growth would include the influence of their external electrical field. Crystal growth from the liquid phase will not show phenomena dependent on an external electrical field and thus cannot duplicate the symmetry found in snowflakes. It is interesting to note that this restriction would not necessarily apply if vibrational processes were the source of symmetry.

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1 Maddox, J *Nature* **313**, 93 (1985)
2 Vicesk, T *Phys Rev Lett.* **53**, 2281 (1984)
3. McLachlan, *Proc natn Acad Sci U S A* **43**, 143 (1957)
4. Tolansky *Nature* **181**, 256 (1958)
5 *J Wash, Acad Sci* **48**, 273 (1958)
6 Zawdski & Papée *Nature* **196**, 568 (1962).
7 Pohl J *appl Phys* **22**, 869 (1951), **29**, 1182 (1958)
8. Nakaya, U *Snow Crystals, Natural and Artificial* (Harvard University Press, 1954)

Source of Precambrian chemical and clastic sediments

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The Nd isotope composition of chemical and clastic sediments surviving from the Archaean provides no evidence for the existence of pre-4,000-Myr continental crust. Either the amounts of such crust were trivial or it was destroyed substantially by 3,800 Myr ago. Nd in major Precambrian banded iron formations was supplied largely by continental weathering, and in this respect the water masses in which they formed were analogous to the modern oceans.

THE oldest surviving vestiges of undisputed continental crust are no older than 3,800 Myr. If a substantial amount of continental crust existed before then, it must have been destroyed and essentially lost from the record. Materials eroded from such an early crust, however, might have contributed to later Archaean sedimentary systems.

Clastic and chemical sediments deposited throughout the Phanerozoic differ from those deposited earlier in Earth history in several important respects. One of the most pronounced differences concerns the apparent absence of banded iron formation (BIF) in the Phanerozoic and its abundance in the Archaean and early Proterozoic[1-3]. In the present-day weathering environment, Fe(II) is readily oxidized to Fe(III), which, because of its low solubility in normal *p*H ranges, suffers little transport. As a consequence, the concentration of Fe in sea water is far too low ($\sim 3\times10^{-6}$ g l^{-1})[4] to produce extensive Fe-rich sediments, such as those once deposited in the Hamersley Basin ($\sim 2\times10^{10}$ kg Fe annually)[5]. At the time of BIF deposition, substantially higher concentrations of Fe may have developed in sea water, possibly in response to more reducing conditions in the oceans[6].

Important changes may have occurred also in the clastic sedimentary record. For example, Taylor and colleagues[7-9] have shown that many Archaean sediments are characterized by relatively low abundances of Th and U and the absence of a Eu anomaly, whereas Proterozoic fine-grained clastic sediments possess both higher Th and U abundances and a pronounced Eu anomaly. These differences have been interpreted to reflect a fundamental change in the character of the exposed continental crust. It remains to be demonstrated, however, whether or not the Archaean clastic sediments that have surived to the present (now found largely in greenstone belts) are representative of the entire sedimentary mass as it existed at those times. If the inferred changes in the chemical character of the continents are indeed real, and not an artefact of selective sampling or sediment preservation, then their implications for the changing nature of continental differentiation are profound.

The question of the provenance of Precambrian chemical and clastic sediments and their implications for the development of the exposed continental crust are investigated here by the measurement of ^{143}Nd abundances in Precambrian sediments. The use of the ^{143}Nd natural radiogenic tracer (α-decay product of ^{147}Sm; $t_{1/2}=1.06\times10^{11}$ yr) derives from the ~40% lower Sm/Nd ratio of the continents compared with the mantle. The uniform Sm/Nd ratio of fine-grained clastic sediments, together with a complementary evolution of ^{143}Nd in the continents and the mantle, renders sediments from old and juvenile crustal sources readily distinguishable, even when these sources have been reworked and recycled in the crust[10-16]. The relationship allows an estimate to be made of the average residence time of the Sm and Nd components in the continents rather than the mantle ($=t_{CR}$, or model crustal residence age)[12]. It is well established[17-20] that the isotope composition of modern seawater Nd reflects a major input from continental sources and that this signature is acquired by chemical sediments presently forming in the ocean basins. On this basis, Precambrian BIFs are expected to acquire their Nd from the water bodies from whch they were precipitated and thereby provide information on the nature of the sources involved.

Sm–Nd isotope data are reported here for Archaean fine-grained clastic sediments from Canada and South Africa and for samples of some classic banded iron formations in Africa, Australia, North America and Greenland. Samples from a late Proterozoic (~450–800-Myr-old) BIF in Brazil have also been analysed because they seem to have all the essential features of the older and better-known examples.

Sample selection and techniques

Fine-grained clastic sediments either temporally or spatially associated with BIF occurrences have been selected both to compare the provenance of coeval chemical and clastic sediments and, together with published data, to investigate the sources of Archaean sediments in general (Tables 1, 2). Specifically included are samples from the ~3,450-Myr-old Fig Tree and Moodies Groups of the Swaziland Supergroup[21-25], the somewhat younger Pongola Supergroup (~2,950 Myr) located south of the Swaziland Supergroup[21,25,26] and the ~2,700-Myr Yellowknife Supergroup of the Canadian Northwest Territories[27-30].

Samples of oxide (±silicate, carbonate) facies banded iron formation have been selected from a number of classic BIFs deposited in the interval ~3,800–600 Myr (Table 3). The oldest samples are from the highly deformed and metamorphosed Isua supracrustal belt in south-west Greenland, formed ~3,750 Myr ago[31-38]. Typically, the iron formation at Isua consists of quartz–magnetite–amphibole–carbonate assemblages[38-40]. Other samples have been selected from the Animikie Basin of Lake Superior (the Biwabik and Negaunee BIFs, deposited ~2,000-Myr ago[41-44]), the Australian Hamersley Basin (~2,400 Myr)[45-47] and the Fig Tree/Moodies Groups[21,48] of the southern African Swaziland Supergroup (~3,450 Myr)[21,49-51]. Samples have also been included from the much younger (800–500 Myr) and less well-known BIF in the Jacadigo Series of Morro do Urucum, Brazil[2,52]. Although this deposit is about an order of magnitude smaller than, for example, the Lake Superior BIF, it seems to be typical of banded iron formations in terms of its mode of occurrence, lithological associations and mineralogy.

Samples of banded iron formation were either sawn into Fe- and quartz-rich bands and analysed separately, or otherwise treated as whole-rock samples in the normal way. All clastic sediment samples were ignited in air to oxidize organic matter. Sample digestion was carried out by conventional techniques using Teflon dissolution vessels. Sm and Nd were separated using a combination of cation-exchange and reverse-phase chromatography as described elsewhere (A. S. Cohen, R.K.O'N. and W. L. Griffin, manuscript in preparation) and mass

spectrometric techniques of isotope analysis followed those described previously[13,53]. The t_{CR} values in Tables 1 and 2 have been calculated only for those samples with $^{147}Sm/^{144}Nd \leq 0.14$.

Results

Clastic sediments. Sm–Nd isotope results for 10 samples of fine-grained clastic sediment collected from greenstone belt associations are presented in Fig. 1 and Table 1. The small range of Sm/Nd ratios ($0.101 < ^{147}Sm/^{144}Nd < 0.125$) is comparable with reported ranges for other Archaean (Table 2) and post-Archaean fine-grained clastic sediments and the particulate loads of modern rivers[11]. Taken together, these results do not show any significant changes in the Sm/Nd ratio of sediments from the Archaean through to the present day, and do not provide evidence for overall changes to more or less acid compositions. However, note that metasediments with $^{147}Sm/^{144}Nd > 0.2$ have been reported from both the Isua and the Malene supracrustal belts of south-west Greenland[54], a ratio that is substantially higher than the more normal values reported by Hamilton *et al.*[13].

The ~3,450-Myr-old Swaziland Supergroup shales have crustal residence ages of 3,500–3,600 Myr for those samples with

Table 1 Sm–Nd isotope analyses of banded iron formations and ancient clastic sediments

Sample	Lithology	Nd(p.p.m.)	Sm(p.p.m.)	$^{147}Sm/^{144}Nd$ ±0.1% ($2\sigma_m$)	$^{143}Nd/^{144}Nd$ $\pm 2\sigma_m$	t_{CR}* (Myr)	ε^t_{Nd}†
Hamersley Basin: 2,400 ± 100 Myr							
120295	BIF whole-rock powder	4.744	1.275	0.1624	0.512022 ± 28	ND	−1.5
120296	BIF whole-rock powder	0.3228	0.08615	0.1613	0.511881 ± 18	ND	−3.9
120301 A	BIF magnetite fragment	3.258	0.7524	0.1396	0.511816 ± 16	ND	+1.6
120301 B	BIF chert fragment	1.254	0.3414	0.1646	0.512105 ± 18	ND	−0.5
103696 A	BIF chert fragment	1.198	0.2694	0.1359	0.511682 ± 18	ND	+0.1
103696 B	BIF chert fragment	0.3674	0.1364	0.2245	0.512841 ± 16	ND	−4.7
103696 C	BIF magnetite fragment	3.700	0.7756	0.1267	0.511491 ± 14	ND	−0.8
103696 D	BIF whole-rock fragment	0.7862	0.2168	0.1667	0.512110 ± 16	ND	−1.1
Fig Tree/Moodies Groups: 3,450 ± 100 Myr							
SL 1	BIF whole-rock powder	1.092	0.3314	0.1835	0.512355 ± 18	ND	+0 3
SL 28 A	BIF whole-rock powder	0.5846	0.1258	0.1301	0.511179 ± 16	3,570	+1.1
SL 28 B	BIF magnetite fragment	0.5138	0.1198	0.1409	0.510955 ± 18	ND	−8.1
SL 583	Fe-shale	3.434	0.7597	0.1337	0.511047 ± 18	3,970	−3.1
SL 9	Shale	4.106	1.200	0.1766	0.512091 ± 14	ND	−1.8
1105	Shale	23.81	4.605	0.1169	0.510863 ± 18	3,570	+0.9
9274	Shale	18.23	3.617	0.1199	0.510975 ± 24	3,510	+1.7
1102	Shale	25.78	4.661	0.1093	0.510694 ± 14	3,560	+0.9
Pongola Supergroup: 2,950 ± 150 Myr							
PM2A	Phyllite	26.40	4.727	0.1082	0.511003 ± 24	3,080	+1.7
PM3A	Phyllite	17.82	3.466	0.1175	0.511320 ± 16	2,890	+4.4
Yellowknife Supergroup: 2,700 ± 100 Myr							
R1	Phyllite	21.79	4.503	0.1249	0.511305 ± 20	3,150	−1.1
R2	Phyllite	28.46	4.748	0.1008	0.511103 ± 22	2,750	+3.4
F63-82	Phyllite	34.13	6.163	0.1091	0.511231 ± 18	2,780	+3.0
Isua Supracrustals: 3,750 ± 50 Myr							
167642	BIF whole-rock powder	1.164	0.3344	0.1736	0.511840 ± 16	ND	−4.5
242573 A	BIF magnetite powder	0.4650	0.1461	0 1898	0.512032 ± 20	ND	−8.6
242573 B	BIF magnetite fragment	1.057	0.2501	0.1430	0.511452 ± 18	ND	+2.9
242573 C	BIF magnetite fragment	0.8122	0.2285	0.1701	0.511806 ± 18	ND	−3.4
242573 D	BIF chert powder	0.5161	0.1300	0.1523	0.511357 ± 18	ND	−3.6
242573 E	BIF chert fragment	0.2654	0.1002	0.2284	0.512658 ± 18	ND	−15.2
242573 F	BIF chert fragment	0.3420	0.1143	0.2020	0.512126 ± 16	ND	−12.7
Animikie Basin: 2,000 ± 100 Myr							
107811	BIF whole-rock powder	34.56	6.884	0.1204	0.511633 ± 14	2,470	0.0
98153	BIF whole-rock powder	7.451	1.103	0.0895	0.511104 ± 16	2,500	−2.4
98154	BIF whole-rock powder	7.674	1.457	0.1148	0.511380 ± 26	2,720	−3.5
98173	BIF whole-rock powder	8.273	1.692	0.1236	0.511676 ± 26	2,490	0.0
Morro do Urucum, Brasil: 650 ± 150 Myr							
URU A	BIF chert fragment	6.455	1.243	0.1164	0.512164 ± 18	1,540	−2.6
URU B	BIF haematite fragment	5.765	1.137	0.1192	0.512162 ± 12	1,590	−2.9
URU C	BIF jasper fragment	21 62	2.795	0.0781	0.512012 ± 16	1,280	−2.4

143Nd/^{144}Nd ratios are normalized to $^{146}Nd/^{144}Nd = 0.7219$.

* t_{CR} model ages calculated assuming mantle evolution of Nd from $\varepsilon^{4,560\ Myr}_{Nd} = 0$ to $\varepsilon^{0}_{Nd} = +10$. Thus:

$$t_{CR} = \frac{1}{\lambda} \ln\left(1 + \frac{0.513151 - {}^{143}Nd/^{144}Nd}{0.2136 - {}^{147}Sm/^{144}Nd}\right)$$

t_{CR} was calculated only for samples with $^{147}Sm/^{144}Nd < 0.14$, for which samples there is a sufficiently large Sm/Nd fractionation relative to depleted-mantle values for the model age to be reliable, assuming a closed-system evolution. ND, not done.

† ε^t_{Nd} is $10^4 \times$the deviation of $^{143}Nd/^{144}Nd$ measured in a sample from the calculated $^{143}Nd/^{144}Nd$ ratio in a CHUR at time t. ε^t_{Nd} values are calculated for the estimated stratigraphic ages of the samples. Analytical errors result in typical uncertainties in ε^t_{Nd} of $< \pm 0.6$. The effect of uncertainty in stratigraphic age on ε^t_{Nd} is illustrated graphically in Fig. 2.

$^{147}Sm/^{144}Nd$ ratios <0.14 (Tables 1, 2). Samples SL9 and SL583 yield t_{CR} ages and ε^t_{Nd} values (Table 1) inconsistent with the results obtained for the other shales: it may be significant both that the $^{147}Sm/^{144}Nd$ ratio found for SL9 is unusually high (0.177) and that SL583 differs from all other samples in containing >50% Fe_2O_3. The two phyllites from the Pongola Supergroup have $^{147}Sm/^{144}Nd$ ratios typical of fine-grained clastics and t_{CR} values of 2,900 and 3,100 Myr, respectively. For both supergroups, t_{CR} is indistinguishable from the estimated depositional age. Such results indicate that the precursors of the Swaziland and Pongola Supergroup sediments, as for most of the Archaean fine-grained clastic sediments so far analysed (refs 10, 13, 16 and Cambridge unpublished data), had an unresolvable pre-depositional crustal residence and have been derived from essentially contemporary additions to the continental crust presumably associated with the formation of the greenstone belts. However, the normal $^{147}Sm/^{144}Nd$ ratio for these sediments indicates that if volcanic additions were a major source, then they must have possessed low Sm/Nd ratios similar to calc-alkaline volcanics. One of the three metasediments from the ~2,700-Myr-old Yellowknife Supergroup yields a rather higher t_{CR} age of 3,200 Myr. Although penecontemporaneous volcanics in this greenstone belt may have been major contributors to the sediments, there seems to be a requirement for a contribution from a ≥3,000-Myr-old source. Whole-rock Rb–Sr ages of 3,100 Myr, and detrital zircons with ages ≤3,500 Myr, have been reported from the Slave craton[55–58], and sialic material identified petrologically in the Supergroup sediments[27] may be derived from this older basement.

Banded iron formation. Sm–Nd isotope results for 25 samples of BIF are presented in Table 1. These samples include normal powdered whole rocks and, to assess the effects of metamorphism and/or variable provenance of Nd, small samples (≤1 g) cut from individual bands. The $^{147}Sm/^{144}Nd$ ratios of the BIF reported here are more variable than those of fine-grained clastic sediments. It is well established that fine-grained clastic sediments of all ages exhibit a restricted range of Sm/Nd with $0.09 < {}^{147}Sm/^{144}Nd < 0.16$ (mean = 0.115)[10–16,25,54,59,60], which contrasts with the larger range for BIF reported in Table 1, where $0.08 < {}^{147}Sm/^{144}Nd < 0.23$. These results confirm the study of rare-earth elements in BIF made by Fryer[61], which yielded a range $0.09 < {}^{147}Sm/^{144}Nd < 0.19$. t_{CR} values for BIF have only been calculated for whole-rock samples with $^{147}Sm/^{144}Nd <$ 0.14, that is, Sm/Nd ratios considerably lower than model mantle values. The concentration of Nd in BIF is usually <6 p.p.m. (Table 1; ref. 61), but reaches 35 p.p.m. for one of the Lake Superior samples.

In general, the effects of metamorphism on the Sm–Nd isotope systematics of fine-grained clastic sediments are minimal, which renders information concerning their provenance readily accessible. However, the response of the Sm–Nd system in BIF to metamorphism has not been investigated previously, and, if significant, would limit severely the provenance information which can be elicited. The Isua BIF provides an ideal opportunity to assess this possibility because the Isua region was subjected to high-grade metamorphism in the upper amphibolite facies ~3,700-Myr ago, and was further metamorphosed at ~2,500 and 1,600 Myr[36,37].

Specimen 242573 (Table 1) consists of alternating 1–10-mm bands of magnetite or quartz, from which six samples were taken: two are bulk Si-rich and Fe-rich powders respectively, and four are individual adjacent fragments removed from two separate bands. The results obtained (Table 1) are inconsistent with evolution of ^{143}Nd in a reservoir with an initially uniform $^{143}Nd/^{144}Nd$ ratio that became a closed system 3,700 Myr ago. $^{147}Sm/^{144}Nd$ and $^{143}Nd/^{144}Nd$ are correlated positively and indicate mobility of Sm and Nd since 3,700 Myr ago, most probably related to the young metamorphic events identified previously in the Isua area. The perturbation of the Sm–Nd system more than 1,000 Myr after the deposition of the Isua BIF means that the computed initial $^{143}Nd/^{144}Nd$ ratios (expressed as ε^t_{Nd} values; see Table 1 legend) are not valid estimates for these samples at time t, and suggests that in general ε^t_{Nd} values calculated from larger whole-rock BIF samples may be more reliable.

Two whole-rock samples from the ~3,450-Myr-old Fig

Table 2 Published Sm–Nd isotope analyses of ancient clastic sediments, >2,000 Myr old

Sample	Source	$^{147}Sm/^{144}Nd$	$^{143}Nd/^{144}Nd$ $\pm 2\sigma_m$	t_{Strat} (Myr)	t_{CR} (Myr)	Refs
Fig tree	Fig Tree Shales	0.1322	0.511193±20	3,350–3,540	3,630	10
KH 44	Australia	0.1220	0.511177±26	2,700–3,000	3,260	10
WC 3	Australia	0.1190	0.510977±24	2,700–2,800	3,470	16
PG 7	Australia	0.1124	0.510609±12	>3,300	3,790	16
242579	Isua Supracrustals	0 1026	0.510381±14	3,750	3,770	13
242582	Isua Supracrustals	0.1055	0.510472±14	3,750	3,740	13
242576	Isua Supracrustals	0.1437	0.511403±26	3,750	3,770	13
242565	Malene Supracrustals	0.1103	0 511122±18	2,600–3,000	2,970	13
242562	Malene Supracrustals	0.0945	0.510738±22	2,600–3,000	3,070	13
81062	Baltic Shield	0.1173	0.510987±14	2,800	3,390	Our unpublished
81064	Baltic Shield	0.1217	0 511301±24	2,800	3,050	data

The original grain size of the metamorphosed samples is generally unknown
* Model ages calculated as for Table 1.

Table 3 Summary geology of sampled banded iron formations

Deposit	Age (Myr)	Mass (kg)*	Thickness (m)	Area (km^2)	Notes	Refs
Isua	3,750	2×10^{12} (ore only)	200 (ore only)	—	Metamorphosed—amphibolite grade. Original size unknown, may be thickened tectonically	31, 35, 38–40
Fig Tree	3,350–3,540	10^{12}	Generally 6–40, <300	—	Low-grade metamorphism. Many small lenses in clastic sedimentary succession	21, 48, 51
Hamersley	2,300–2,500	10^{17}	<350	100,000	~Unmetamorphosed, T_{max} ~270–310 °C. Five major bands, total 1,000 m thick	45, 47
Lake Superior	2,100–1,850	10^{16}	<240	220,000	Metamorphosed chlorite to sillimanite grades at ~1,860 Myr	41–43
Urucum	~600	10^{15}	>300	>1,000	Unmetamorphosed. Contains lenses of manganese oxides, glaciogenic clastics	52

* Mass data from ref. 2.

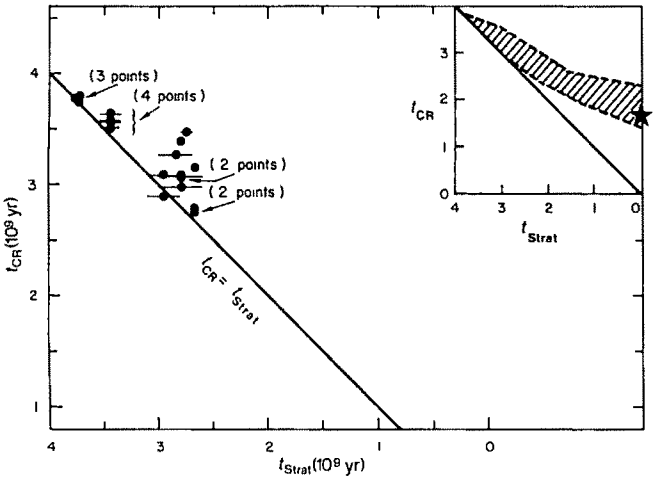


Fig. 1 Comparison of estimated stratigraphic ages (t_{Strat}) and crustal residence ages (t_{CR}) calculated from Sm–Nd isotope analyses (Tables 1, 2). Main graph, Archaean fine-grained clastic sediments. Pre-4,000-Myr contributions to these Archaean sediments cannot be discerned. Most crustal residence ages are ≤200 Myr greater than stratigraphic ages. Inset diagram shows that this relationship does not extend into the Phanerozoic; asterisk represents the mean value for fluvial fine-grained present-day clastic sediments (ref 11); shaded region, world-wide fine-grained clastics.

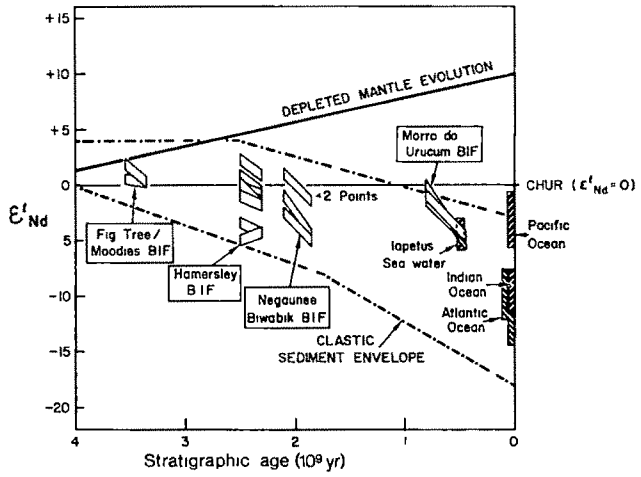


Fig. 2 Banded iron formations. ε^t_{Nd} values for samples of BIF, sea water and ferromanganese deposits from modern oceans and metalliferous sediments deposited in Iapetus. ε^t_{Nd} values are 10^4 times the deviation of initial $^{143}Nd/^{144}Nd$ ratios in a sample from those in a CHUR at time t. Note that all samples have ε^t_{Nd} in the range of coeval clastic sediments. The estimated ε^t_{Nd} of the depleted mantle excludes a dominant mantle supply of Nd into the majority of BIFs. Isua BIF samples and sample SL28B omitted (see text).

Tree/Moodies Groups BIF, which have been metamorphosed to no more than greenschist facies, yield $+0.3<\varepsilon^t_{Nd}<+1.1$ (Table 1), although a small fragment from an individual magnetite band yields a very different ε^t_{Nd} value of -8 and again suggests small-scale mobility of rare-earth elements. Six single fragments and two whole rocks from the virtually unmetamorphosed Hamersley Basin BIF yield a range of values with $-4.7<\varepsilon^t_{Nd}<+1.6$. The four whole-rock samples from the Animikie Basin BIF yield a similar range of ε^t_{Nd} values ($-3.5<\varepsilon^t_{Nd}<0$), and crustal residence ages between 2,500 and 2,700 Myr. The Morro do Urucum BIF is little metamorphosed and yields $-2.9<\varepsilon^t_{Nd}<-2.4$. The calculated ranges in ε^t_{Nd} values for a single BIF are comparable with those in sea water from a present-day ocean basin (Fig. 2), and may reflect real variations in Nd isotope composition in the water masses concerned, or the effects of post-depositional mobilization of Sm–Nd. Although the effects of metamorphic redistribution are severe on the scale of the centimetre banding in the high-grade Isua BIF, they are much less so in the lower-grade BIF. Estimates of the ε^t_{Nd} values for the water masses from which Hamersley, Animikie and Urucum BIF were deposited are based consequently on the results from whole-rock samples, but it must be conceded that the small differences between samples could reflect either initial heterogeneities in the water masses and/or post-depositional redistribution of Sm and Nd.

Chemical and clastic sediments

Note that of 18 fine-grained Archaean clastic sediments with $^{147}Sm/^{144}Nd<0.14$ for which reliable crustal residence ages can be calculated, 13 possess t_{CR} and estimated stratigraphic (t_{Strat}) ages that agree within ~200 Myr (Fig. 1). Notwithstanding the uncertainty in the correct choice of depleted-mantle models used to calculate the t_{CR} values, this observation supports the notion that the Archaean sediment source was dominated by juvenile material that had not resided as part of the continental crust for more than ~200 Myr before its erosion and deposition. No major input of relatively old crust into clastic sediments can be observed until <3,000 Myr ago. Note also that if significant quantities of substantially older crust (that is, >4,000 Myr) existed, then it had either failed to survive by 3,800–3,500 Myr ago, or else sampling to date has not included clastic sediments derived from this material, and they remain undiscovered. The latter explanation would leave open the possibility that such an ancient source supplied materials to the oceans and might occur in chemical sediments.

The $^{143}Nd/^{144}Nd$ ratios measured for modern seawater and pelagic sediments are similar to the global characteristics of the particulate load of the world's major rivers and atmospheric dusts[11,17–20]. Significant shifts to more positive ε^0_{Nd} values in Pacific sea water and ferromanganese deposits may result from a juvenile Nd component with $\varepsilon^0_{Nd}\approx 10$, such as might be supplied from the oceanic ridges or the relatively youthful continental margins around the Pacific (Fig. 2). Chemical precipitates forming in present-day oceans as manganese nodules or Fe-rich sediments associated with spreading-ridge axes[17] have acquired Nd isotope compositions in the ranges exhibited by the water bodies in which they have formed. It is a reasonable expectation, therefore, that the Precambrian iron formations will have similarly sequestered Nd from the water bodies in which they formed. Hooker *et al.*[53] have previously derived estimates for the Nd isotope composition of Iapetus sea water from ~490-Myr-old metalliferous sediments in the Southern Uplands of Scotland. Even though rare-earth mobility may have accompanied burial and diagenesis, it is possible to reconstruct the ε^t_{Nd} of the water bodies concerned from Sm–Nd analyses of BIF, as long as rare-earth immobility has prevailed on the scale of sampling from close to the time of sedimentation. It has been demonstrated above, particularly in the case of Isua, that such immobility cannot be presupposed for BIF. However, less metamorphosed BIF and/or larger scales of sampling have yielded results from which details of the provenance of their rare-earth elements can be deduced.

The principal question is whether some special source of Nd, and therefore possibly of Fe, must be invoked to explain the observations on BIF, or whether average crustal sources such as supplied the clastic sedimentary mass during most of crustal history have prevailed. This situation can be assessed using those measurements on BIF thought to yield reliable estimates of ε^t_{Nd}, by comparing them with similar observations for the clastic sedimentary mass and for modern and Iapetus ocean water (Fig. 2).

We find that ε^t_{Nd} values for the Fig Tree BIF are comparable with those of the contemporary clastic sediments from the Swazi-

land Supergroup, and close to both the chondritic uniform reservoir (CHUR) model or a depleted-mantle model at that time. Thus, the water body from which the Fig Tree BIF was precipitated seems to have received its Nd from a juvenile source whose average previous crustal residence time had not exceeded ~200 Myr. The early Proterozoic BIFs from Lake Superior and Hamersley have variable, mostly negative ε^t_{Nd} values, indistinguishable in this respect from their contemporary fine-grained clastic sediments. Similarly, the Morro do Urucum BIF has ε^t_{Nd} and t_{CR} estimates similar to those for coeval clastic sediments but which display a bias towards slightly more juvenile Nd, as does modern Pacific sea water. In all the post-Archaean BIFs, the Nd could have been derived from an analogue of modern sea water, dominated by Nd typical of that of the average exposed continental crust (as recorded by fine-grained clastic sediments) but also containing a relatively juvenile component. In no case does Proterozoic BIF show evidence for a dominantly juvenile mantle source for its Nd, so that a special source involving unusual amounts of volcanic Nd input seems to be excluded. A direct mantle source of Nd for Archaean BIF cannot be excluded, however, because of the similarity of Nd in the crustal and mantle reservoirs at that time. There is also no evidence for the derivation of a major component of BIF Nd from pre-3,500 Myr continental sources. If relatively ancient continental crust (>4,000-Myr-old) existed at times of BIF deposition, then not only is its contribution to the clastic sedimentary mass unresolvable, but any contribution it made to major water bodies was masked by inputs from more juvenile crustal sources.

The results of this investigation directly address the source of Nd in BIF and demonstrate the importance of continental sources of Nd in the oceans during the Proterozoic as well as at the present day, but leave open to conjecture the source of Fe. The Fe/Nd ratio of the continental crust and typical clastic sediments is $\sim 1.5 \times 10^3$ and in the range 10^3–10^4 for river waters, compared with 10^5 for typical Hamersley BIF containing 35% Fe and 3 p.p.m. Nd. Both Fe and Nd in Precambrian BIF could have been supplied from continental sources if estuaries offered less impediment to iron transfer from rivers to the open ocean than they do today[62–66]. Although the alternative possibility, a submarine hydrothermal source of Fe in BIF (but not for Nd) is appealing[6], it is difficult to evaluate. The Fe/Nd ratio of present-day submarine hydrothermal vent waters is poorly known but apparently high (10^5–10^6)[67–69], and this was probably always the case. Therefore, if the Fe in BIF was derived from such a source, its Nd budget would probably have been dominated by continental Nd in the surrounding sea water, consistent with observations on Nd in modern metalliferous sediments and hydrogenous deposits[7]. In the absence of a more effective tracer for hydrothermally derived iron, it is necessary to consider iron mass-balance. The submarine hydrothermal supply of Fe to the present oceans is estimated to be $\leq 10^{10}$ kg yr^{-1} (ref. 70), yet it is claimed that the Hamersely BIF alone required $\geq 10^{10}$ kg yr^{-1} (ref. 5). On the present limited evidence, a major component of Fe derived from the continents may be required to satisfy the iron mass-balance, unless the hydrothermal Fe input was overwhelmingly greater than the present one.

Conclusions

First, Archaean clastic sediments sampled so far were derived from precursors with short previous histories of crustal residence, generally no more than ~200 Myr. Second, Nd in oxide-facies BIF was derived from source material indistinguishable from that supplying the clastic sedimentary mass. A marine source with a Nd budget similar to that of modern oceans is possible for all BIFs, and a dominantly mantle source is excluded for the post-Archaean varieties. The possibility that Fe may also have been derived largely from continental weathering is not excluded. Third, the input of significant amounts of pre-4,000-Myr clastic material into post-3,800-Myr sediments is excluded by the present data. If continental crust existed in significant quantities before 4,000 Myr, then its erosion products were not incorporated into the surviving Archaean sediments and its signature occurs in neither banded iron formations nor, presumably, the water masses from which they were precipitated. Finally, the similarity throughout Earth history of Nd in coeval fine-grained clastic sediments and BIF, and hence between coeval clastic sediments and ocean water, implies that Archaean clastic sediments may be representative of contemporary crust exposed to erosion as are present-day river sediments.

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1 Cloud, P *Geol. Soc S Afr Annexure* **79**, 1-33 (1976)
2 James, H L in *Iron-Formation Facts and Problems* (eds Trendall, A F & Morris, R. C) 471-490 (Elsevier, Amsterdam, 1983)
3 Walker, J C G *et al* in *Earth's Earliest Biosphere* (ed Schopf, J W) 260-290 (Princeton University Press, 1983)
4 Riley, J P & Chester, R *Introduction to Marine Chemistry* (Academic, London, 1971)
5 Ewers, W. E in *Iron-formation Facts and Problems* (eds Trendall, A F & Morris, R C) 491-512 (Elsevier, Amsterdam, 1983)
6 Veizer, J in *Earth's Earliest Biosphere* (ed Schopf, J W) 240-259 (Princeton University Press, 1983)
7 McLennan, S M & Taylor, S R *Nature* **285**, 621-624 (1980)
8 McLennan, S M & Taylor, S R *J Geol* **90**, 347-361 (1982)
9 Taylor, S R & McLennan, S M *Phil Trans R. Soc.* **A301**, 381-399 (1981).
10 McCulloch, M T & Wasserburg, G J *Science* **200**, 1003-1011 (1978)
11 Goldstein, S L, O'Nions, R K & Hamilton, P J *Earth planet Sci Lett* **70**, 221-236 (1984)
12 O'Nions, R K, Hamilton, P J & Hooker,.P J *Earth planet Sci Lett* **63**, 229-240 (1983)
13 Hamilton, P J, O'Nions, R K, Bridgwater D & Nutman, A P *Earth planet Sci Lett* **62**, 263-272 (1983)
14 Miller, R G & O'Nions, R K *Earth planet Sci Lett* **68**, 459-470 (1984)
15 Frost, C D & O'Nions, R K *Nature* **312**, 53-56 (1984)
16 Allègre, C J & Rousseau, D *Earth planet Sci Lett* **67**, 19-34 (1984)
17 Goldstein, S L & O'Nions, R K *Nature* **292**, 324-327 (1981)
18 O'Nions, R K, Carter, S R, Cohen, R S, Evensen, N M & Hamilton, P. J *Nature* **273**, 435-438 (1978)
19 Piepgras, D J, Wasserburg, G J & Dasch, E J *Earth planet Sci Lett* **45**, 223-236 (1979)
20 Piepgras, D J. & Wasserburg, G J *Earth planet Sci. Lett* **50**, 128-138 (1980)
21 Tankard, A J *et al Crustal Evolution of Southern Africa* (Springer, Berlin, 1982)
22 Eriksson, K. A *Precamb Res* **12**, 141-160 (1980)
23 Condie, K. C, Macke, J E & Reimer, T O *Bull geol Soc. Am* **81**, 2759-2776 (1970)
24 Reimer, T O. *Sediment Geol* **7**, 263-282 (1972)
25 McLennan, S M, Taylor, S R & Kroner, A *Precamb Res* **22**, 93-124 (1983)
26 Hegner, E *et al Earth planet Sci Lett* **70**, 267-279 (1984)
27 Henderson, J B *Bull geol Surv Can* **246**, 1-62 (1975)
28 McGlynn, J C & Henderson, J B in *Variations in Tectonic Styles in Canada* (eds Price, R A & Douglas, R J W) 505-526 (Geol Ass Can Spec Pap No 11, 1972)
29 Green, D C & Baadsgaard, H *J Petrol* **12**, 177-217 (1971)
30 Frith, R A & Loveridge, W D *Geol Surv Can Pap* No 82-1A, 225-237 (1982)
31 Moorbath, S, O'Nions, R K. & Pankhurst, R J *Nature* **245**, 138-139 (1973)
32. Moorbath, S, Allaart, J N, Bridgwater, D & McGregor, V R *Nature* **270**, 43-45 (1977)
33 Michard-Vitrac, A, Lancelot, J & Allègre, C J *Earth planet Sci Lett* **35**, 449-459 (1977)
34 Hamilton, P J, O'Nions, R K, Evensen, N M, Bridgwater, D & Allaart, J N *Nature* **272**, 41-43 (1978)
35 Nutman, A P, Bridgwater, D., Dimroth, E, Gill, R C O & Rosing, M *Rapp Grøn geol Unders* **112**, 5-22 (1983)
36 Baadsgaard, H *Rapp Grøn geol Unders* **112**, 35-42 (1983)
37 Baadsgaard, H, Lambert, R St J & Krupicka, J *Geochim cosmochim Acta* **40**, 513-527 (1976)
38 Bridgwater, D, Keto, L, McGregor, U R & Myers, J S in *Geology of Greenland* (eds Escher, A & Watt, W S) 8-75 (Geol Surv Greenland, Copenhagen, 1976)
39 Nielsen, B L in *Geology of Greenland* (eds Escher, A & Watt, W S) 460-487 (Geol Surv Greenland, Copenhagen, 1976)
40. Appel, P W U *Precamb Res* **11**, 73-87 (1980)
41 Bayley, R W & James, H L *Econ Geol* **68**, 934-959 (1973)
42 Morey, G B in *Iron-Formation Facts and Problems* (eds Trendall, A F & Morris, R C) 13-67 (Elsevier, Amsterdam, 1983)
43 Haase, C S *Econ Geol* **77**, 60-81 (1982)
44 Lepp, H in *Studies in Mineralogy and Precambrian Geology* (eds Doe, B R & Smith, D K) 265-278 (Geol Surv Am Mem No 135, 1972)
45 Trendall, A F in *Iron-Formation Facts and Problems* (eds Trendall, A F & Morris, R C) 69-129 (Elsevier, Amsterdam, 1983)
46 Ewers, W E & Morris, R. C *Econ Geol* **76**, 1929-1953 (1981)
47 Becker, R H & Clayton, R N *Geochim cosmochim Acta* **40**, 1153-1165 (1976)
48 Beukes, N J *Econ Geol* **68**, 960-1004 (1973)
49 Burger, A J & Coertze, F J *Bull geol Surv S Afr* **58**, 1-46 (1973)
50 Davies, R D, Allsopp, H L, Erlank, A J & Manton, W I *Geol Soc. S Afr Spec Publ* **1**, 576-593 (1970)
51 Hamilton, P J, Evensen, N M, O'Nions, R K, Smith, H S & Erlank, A J *Nature* **279**, 298-300 (1979)
52 Dorr, J van N *Econ Geol.* **68**, 1005-1022 (1973)
53 Hooker, P J, Hamilton, P J & O'Nions, R K *Earth planet Sci Lett* **56**, 180-188 (1981)
54 McLennan, S M, Taylor, S R & McGregor, V R *Geochim cosmochim Acta* **48**, 1-13 (1984)
55 Scharer, U & Allègre, C J *Can J Earth Sci.* **19**, 1910-1918 (1982)
56 Nikic, Z *et al Geol Soc. Am. Spec. Pap* **182**, 169-175 (1980)
57 Krogh, T E & Gibbins, W *Geol. Soc Am Abstr Prog* **10**, 438 (1978)
58 Frith, R, Frith, R A & Doig R *Can J Earth Sci* **14**, 1356-1373 (1977)

59 Nance, W B & Taylor, S R *Geochim cosmochim Acta* 40, 1539-1551 (1976)
60 Nance, W B & Taylor, S R *Geochim cosmochim Acta* 41, 225-231 (1977)
61 Fryer, B J *Geochim cosmochim Acta* 41, 361-367 (1977)
62 Boyle, E A, Edmond, J M & Sholkovitz, E R *Geochim cosmochim Acta* 41, 1313-1324 (1977)
63 Fox, L E & Wofsy, S C *Geochim cosmochim Acta* 47, 211-216 (1983)
64 Fox, L E *Geochim cosmochim Acta* 48, 879-884 (1984)
65 Sholkovitz, E R *Geochim cosmochim Acta* 40, 831-845 (1976)
66 Sholkovitz, E R, Boyle, E A & Price, N B *Earth planet Sci Lett* 40, 130-136 (1978)
67 Michard, A, Albarède, F, Michard, G, Minster, J-F & Charlou, J L *Nature* 303, 795-797 (1983)
68 Edmond, J M *et al Earth planet Sci Lett* 46, 19-30 (1979)
69 Von Damm, K L thesis, Massachusetts Institute of Technology (1983)
70. Bostrom, K *Earth planet Sci Lett* 9, 348-354 (1970)

Transmission and expression of a specific pair of rearranged immunoglobulin μ and κ genes in a transgenic mouse line

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Two genes that code for a hapten-specific immunoglobulin M (IgM) have been introduced into the mouse germ line. The transgenic antibody represents 10-50% of the serum IgM and is expressed on the membrane of B cells. B-cell hybridoma lines show that a negative feedback inhibition of μ and κ transgenic products on the immunoglobulin heavy-chain rearrangement is possible.

EXPRESSION of immunoglobulin genes depends on an accurate rearrangement of DNA to bring several gene segments (for example, variable (V_H), diversity (D_H) and joining (J_H) for the heavy chain and V_L and J_L for the light chain) into juxtaposition[1-3]. When a complete functional reading unit is achieved in one chromosome, no further rearrangements of the second chromosome occur[4], a phenomenon termed allelic exclusion. The mechanism responsible for this peculiarity of the immunoglobulin system is poorly understood. To alter the normal rearrangement process, we introduced rearranged μ and κ genes into the germ line of Swiss albino mice. Germline transmission and tissue-specific expression occur in several genes, including immunoglobulin heavy- and light-chain genes[5-11]. Here we show transmission and expression of genes encoding a complete antigen-specific antibody molecule.

Transgenic line derivation

The plasmid, microinjected as circular DNA, contains three genes (Fig. 1*a*); rearranged μ and κ genes encoding an IgM molecule with anti-trinitrophenyl (TNP) specificity (Sp6 in ref. 12) and a bacterial gene for neomycin resistance, the latter under control of the simian virus (SV)40 promoter/enhancer. In gene transfer of B-cell myeloma lines, all the genes of this plasmid are functional[13].

DNA isolated from the tails of 5 surviving mice obtained from 13 implanted embryos was hybridized to plasmid pBR322 DNA; in one case (209/F_0, Fig. 1*b*) we obtained weak but significant hybridization. Mating the 209/F_0 male to a random-bred female yielded 10 offspring mice; the DNA from one of them (F_{1a}) hybridizes ~10-fold more strongly with pBR. Now we have obtained 40 offspring of the 209/F_0 founder, of which three carry the gene. We conclude that 209/F_0 has chimaeric spermatogonia. Male and female offspring of the F_{1a} male show ~50% segregation (a total of $31^+/54$ in matings with random-bred Swiss Albino ICR/Z, BALB/c and A/J females), as is expected if the plasmid DNA is integrated in one autosomal chromosome.

The presence of pR-HL_{TNP} sequences was checked in a Southern blot analysis where F_0, F_{1a} and F_2 tail DNA was cut with *Bgl*II and *Pst*I restriction enzymes and probed with an α_1-globin-containing plasmid. Small 2.5- and 3.5-kilobase (kb) *Pst*I fragments and a large 11-kb *Bgl*II fragment were revealed, as predicted from the behaviour of the pR-HL_{TNP} plasmid (Fig. 1*c*, bands marked T). By comparing the intensity of the 'T' bands with endogenous α-globin bands (Fig. 1*c*), we estimate that there are about one and four copies of pR-HL_{TNP} per diploid genome in 209/F_0 and F_{1a} (or F_2), respectively. This can be explained by assuming that in F_{1a} (or F_2) every tail cell carries ~4 copies of pR-HL_{TNP} (a number confirmed in B hybridomas), but in 209/F_0, an average of one cell in four has the same copy number. An analysis of B-cell hybridomas obtained from 209/F_0 lymph node cells also reveals a mosaic distribution of the transfected gene—only 2 out of 19 hybrids analysed expressed Sp6-like IgM.

Specific IgM

Serum immunoglobulin determinations in transgenic and control mice are summarized in Table 1. All transgenic mice have an elevated anti-TNP/sheep erythrocyte (SRBC) agglutination titre correlating with an elevated expression of an IgM-associated idiotypic determinant characteristic of Sp6 IgM. There are also small but significant amounts of Sp6 IgM in the F_0 serum. Quantitative radioimmunoassays, using monoclonal anti-μ for coating and radiolabelled monoclonal anti-μ or anti-idiotypic antibodies to reveal total and idiotypically positive (Id^+) IgM, showed between 140 and 420 μg Id^+-IgM and 380-1,200 μg total IgM in F_2 and M_2 (mating 2) transgenic adult sera. That is, there is a 1,000-4,000-fold increase of Id^+-IgM and a 2-5-fold increase of total IgM in transgenic F_2 or M_2 mice over their normal litter mates. The relatively small increase of total IgM may be insignificant or may be unrelated to the transfection, as similar increases do not occur in the sera of transgenic animals resulting from matings of F_{1a} with BALB/c− and A/J females. We found similar levels of protein A binding, κ-light-chain-bearing immunoglobulins (mostly IgG) and λ-light-chain levels in transgenic and control mice (Table 1). Therefore, the introduced IgM does not interfere dramatically with the normal levels of the intrinsic immunoglobulin classes.

To elucidate further the cells responsible for the high Id^+-IgM concentrations in the serum, we measured immunofluorescence of spleen cells (control values of pR-HL_{TNP}-negative litter mates in parentheses). Approximately 42% (41%) of the cells were stained with an anti-μ reagent and ~27% (3%) and 22% (2%) were double stained with TNP-bovine serum albumin and anti-idiotype, respectively. This shows that spleen B cells express membrane-bound Id^+-IgM. To confirm this expression, we

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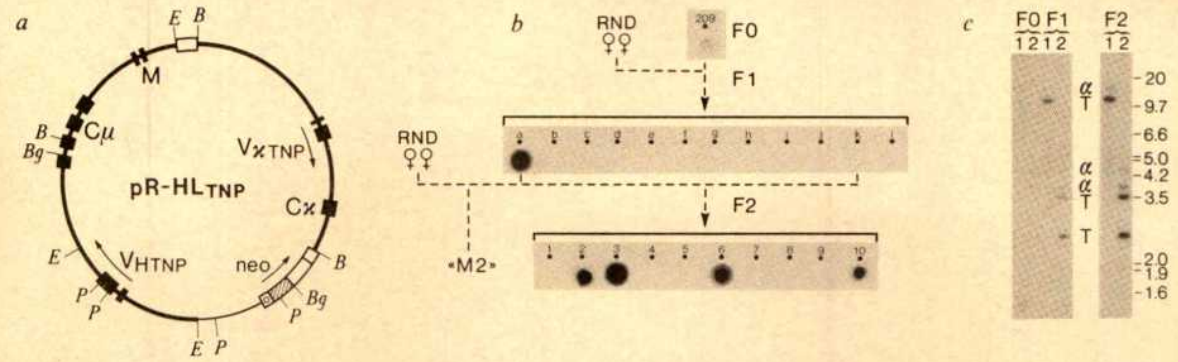


Fig. 1 Transformation of mice with pR-HL$_{TNP}$ DNA. *a*, Schematic representation of the 30-kb DNA used for microinjection, bearing both the functionally rearranged κ light-chain (V_{TNP} and $C_κ$) and heavy-chain (V_{TNP} and $C_μ$) genes, the combined products of which give rise to an antibody specific for the hapten TNP (see refs 12, 13). Thick line, mouse genomic DNA; thick bars, exons; open bars and hatched bars, SV40 and neomycin resistance sequences from the vector pSV2-neo[26]; thin line, plasmid DNA. Restriction sites: E, *Eco*RI; B, *Bam*HI; only the *Bgl*II (Bg) and *Pst*I (P) sites indispensable for the interpretation of the Southern blot presented in *c* are shown. *b*, The pR-HL$_{TNP}$ transgenic mouse line analysed by dot-blot hybridization. Thirteen fertilized eggs that survived pronuclear microinjection of 20–100 molecules of pR-HL$_{TNP}$ were implanted in the oviduct of a recipient female (see ref. 27). From the five offspring, one contained pBR-hybridizing sequences. The F_1 progeny was generated by mating mouse 209 with a random-bred female. The F_2 progeny resulted from *inter se* cross of mouse F_{1a} and the negative female F_{1k}. Repeated crosses of F_{1a} with other females of the same or different strains lead to the 'M_2' (mating 2) generation (to date, 54 individuals) which was screened either by dot or Southern blot hybridization or serum analysis (see Table 1). Conditions for dot-blot: 2 μg DNA from the tail tip[28] was spotted on nitrocellulose filters (loading buffer, 0.5 M NaOH, 1.5 M NaCl (Schleicher & Schuell); 0.45 μm; manipulations essentially as in ref. 29). ^{32}P-nick translated pBR327 (6×10^8 d.p.m. μg^{-1}) was used to probe for the presence of exogenous plasmid sequences in the genomic DNA. *c*, Determination of relative copy number and structural organization of the exogenous pR-HL$_{TNP}$ DNA in the transgenic line 209. The DNA was analysed by Southern blots (details in ref. 28). Each lane contains 1 μg of restricted genomic DNA (1, *Bgl*II; 2, *Pst*I). The hybridization probe was a pBR recombinant clone containing 2.7-kb mouse $α_1$-globin DNA[30] labelled with ^{32}P-CTP. Bands resulting from hybridization with the pBR moiety of pRHL$_{TNP}$ are indicated with T; α indicates the fragments which contain α-globin gene sequences and which serve as an internal control (see text).

made B-cell hybridomas, using as fusion partner the Sp2/0-Ag14 line[14].

B-cell hybridomas

Lipopolysaccharide (LPS)-stimulated lymph node cells of the F_{1a} mouse yields 32 of 72 wells with B-cell hybridoma growth, 30 of which contain anti-TNP-specific Id$^+$-IgM in the supernatant. Supernatants of radiolabelled cultures of independent recloned hybridoma lines were analysed by SDS and isoelectric focusing (IEF) polyacrylamide gel electrophoresis (PAGE) under reducing conditions (Fig. 2; Table 2). Only those hybrids originating from F_{1a} lymph node cells and listed in Table 2 will be discussed here in detail.

For 5 of the 11 F_{1a} hybrids analysed (for example, C5-20-21 and C5-20-22; Fig. 2) a doublet of light-chain-like bands, the weaker of which co-migrated with the Sp6 light chain, was seen after SDS–PAGE. The other six lines (for example E5-28-48 in Fig. 2) show only one strong band at the Sp6 light-chain position. To demonstrate that low concentrations of κ6 are co-expressed with newly rearranged light chains, IEF–PAGE was used. Although κ6 is not seen easily (Fig. 2), it is visible in the original autoradiogram and all lines express it in addition to a cell-line-specific light chain (Fig. 2, arrows). Light chains are easily identified in IEF–PAGE under reducing conditions as isolated bands (heavy chains always run as a series of closely spaced bands). A series of at least three bands characteristic of the Sp6 μ-heavy chain is expressed at varying intensity by all hybrids analysed except H5-35. In addition, in some hybrids, a new set of bands is seen (Fig. 2), which suggests that there is co-expression of μ-heavy chains. This co-expression was confirmed by the subclone C5-20-16, which has lost the pR-HL$_{TNP}$ DNA (not shown) together with the κ6 light chain and idiotype expression, but which synthesizes an IgM with a 20-fold lower TNP/SRBC agglutination titre (Fig. 2; Table 2). Surprisingly, the IEF-bands co-migrating with Sp6 μ-chain are preserved in this line.

Sp6-encoded RNA

To confirm the expression of Sp6 μ- and κ-chains, we performed Northern blot analysis of cytoplasmic hybridoma and Sp6 RNA using $V_μ6$ and $V_κ6$ DNA probes (Fig. 3, Table 2). All lines express the expected 2,4- and 1,2-kb RNA when probed with $V_μ6$ and $V_κ6$, respectively. To quantify the fraction of μ and κ RNA associated with $V_μ6$ and $V_κ6$ variable regions, we performed RNA dot-blot experiments. RNA was blotted on nitrocellulose filters at different concentrations and equivalent samples were hybridized to radiolabelled probes specific for the four elements $V_μ6$, μ constant region ($C_μ$), $V_κ6$ and $C_κ$. The radioactive signals were evaluated quantitatively by scintillation counting of the corresponding excised filter portions (Fig. 3). In all four hybridomas, the intensity of the $V_κ6$ signal is about one-quarter of that of Sp6, whereas the $C_κ$ signal is 1.4–4.6 times stronger than that of Sp6. Thus there is a 6–20-fold excess of $C_κ$ over $V_κ6$ in the four hybridomas analysed. We conclude

Table 1 Immunoglobulin levels of transgenic mice

Serum	TNP/SRBC agglutination titre	Idiotype (μg ml^{-1})	IgM (μg ml^{-1})	Protein A binding (mg ml^{-1})	λ ratio Id$^+$/Id$^-$
F_0	30	0.2	150	2.7	ND
F_{1a}	5,000	200	1,000	2.7	ND
$F_{2/3}$	8,000	220	460	2.0	
$F_{2/6}$	8,000	420	1,200	2.5	1
C2+C4 pool (F_2 litternates)	<10	<0.1	240	1.3	
M_2/40					
41	3,000	140	380	1.0	
42					1
M_2/43+44 pool (M_2 littermates)	<10	<0.1	280	2.0	

Radioimmunoassays were performed by incubating serially diluted sera into antibody-coated 96-well polyvinyl plates. To measure idiotypic and IgM titres, plates were coated with a monoclonal anti-mouse μ antibody (C2-23; ref. 23), bound material was revealed with a ^{125}I-labelled anti-idiotypic monoclonal antibody (20-5; obtained from P.A. Cazenave) or a second, anti-mouse μ antibody (M41; ref. 23). Absolute amounts were obtained by comparison with purified Sp6 IgM. To measure protein A binding material, plates were coated with protein A and bound material was revealed with an anti-mouse κ monoclonal antibody (187.1; ref. 24). Reference material came from C57BL/6 serum purified on a protein A column. Relative amounts of λ-chains in sera of transgenic against normal mice were measured by coating the plates with an anti-mouse λ antibody (L22.14.4; ref. 25) and revealing bound material with the same ^{125}I-labelled antibody. Agglutination titres on TNP-coupled SRBC (TNP/SRBC) were enhanced with C2-23 antibody. ND, not done.

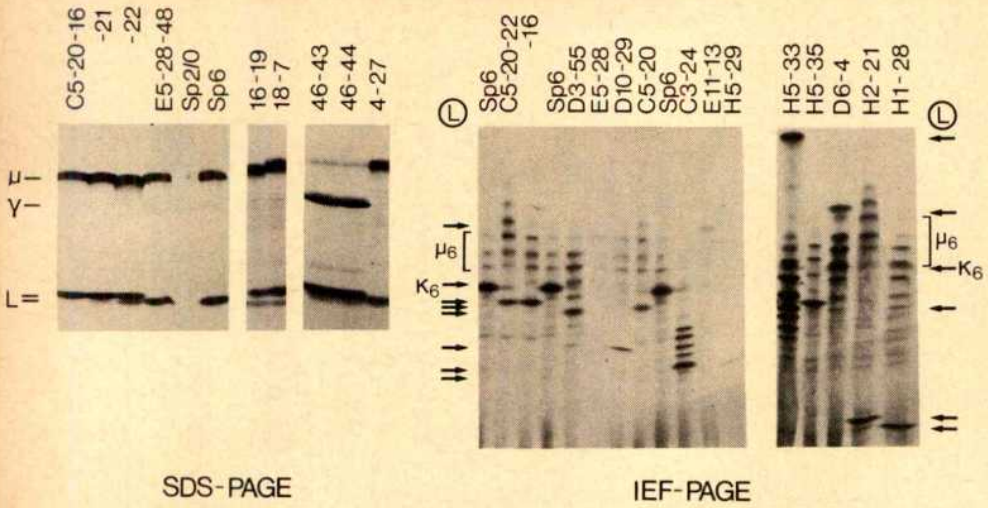


Fig. 2 Separation of reduced immunoglobulin chains. ^{14}C-labelled culture supernatants were obtained and analysed by SDS and IEF-PAGE as described previously[12]. Arrows indicate the positions of endogenously rearranged light chains (IEF-PAGE). Supernatants of lines 16-19, 18-7, 46-43, 44 and 4-27 come from a different fusion and are included to demonstrate co-expression of μ6- and γ-chains (46-43, 44) and of λ1- and κ6-chains (18-7). Antibodies from these lines are TNP- and idiotype-positive.

that, at least in the four hybridomas analysed, the Sp6-derived light chain is expressed at about one-tenth of the level of the second (endogenous) κ light chain. A similar analysis of V_μ6-carrying μ RNA reveals that in C5-20, ~40%, and in the other hybrids, ~75% of μ RNA might be derived from Sp6.

Heavy-chain locus rearrangements

In 7 out of 11 hybrids, there is no evidence for co-expression of a μ-chain derived from Sp6 with an endogenously derived μ-chain (Table 2). To exclude chromosome loss and to test for rearrangements in the J_H region, we hybridized the *Pvu*II- and *Xba*I-restricted DNA from all clones with a purified J_H3/J_H4 (*Bam*HI/*Eco*RI) probe (Fig. 4*a*; Table 2), with DNA from the Sp2/0 fusion partner and BALB/c liver as controls.

The Sp2/0 band is present in the DNA of all cloned hybridomas except D10-29 and H5-33. The hybridomas share the following properties: they show one strong and two weaker pR-HL_{TNP}-derived bands (dots, Fig. 4*a*); they have lost the germline J_H band of 4.8 kb (*Pvu*II) and 3.7 kb (*Xba*I) but have replaced it with one or two weaker bands of varying size. We conclude that all hybrids have retained both the chromosome carrying the pR-HL_{TNP} DNA and also either one or both chromosomes encoding the heavy-chain genes (Table 2).

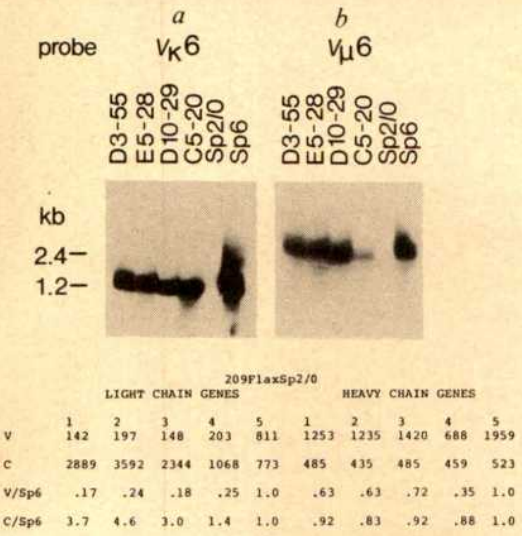
DNA of the hybrid cells, cut with *Pvu*II and *Xba*I restriction enzymes, were also hybridized to two different 5′ *D*-segment probes (Fig. 4*b*; Table 2). If a heavy-chain gene is rearranged to become a complete *V–D–J* unit, the intervening DNA containing the *D* segment is deleted and can no longer be detected with 5′ *D* probes[4]. The DNA of the Sp6 line has two rearranged J_H loci[15], both of which are of the *V–D–J* type, as no band is hybridized with the *D* probes (Fig. 4*b*). The Sp2/0-fusion parent has one rearranged J_H locus in the *D–J* configuration, as most of the germline *D* bands are revealed.

The same rearranged band which has been revealed with the *J3/J4* probe at ~17 kb (*Pvu*II) is seen also with the *D* probe in H10, D3-55 and E5-28. Furthermore, as seen in the DNA cut with *Pvu*II, both *J3/J4*-containing bands of E5-28 (22 and 17 kb) and D10-29 (11 and 6.5 kb) hybridize with the *D* probe. In the E5-28 line, the 22-kb DNA (*Pvu*II) that contains *J3/J4* is not seen easily when hybridized to the *D* probe, it is better visualized in the 3.8 kb band appearing after *Xba*I digestion (the 17-kb *Pvu*II band is hidden by other bands in this digest). Thus, in the E5-28 and D10-29 lines, rearrangements have stopped at both heavy-chain loci at the *D–J* stage, and no endogenous μ-chain could be made. In Table 2 the number of *J* and *D* rearrangements are given for all hybrids analysed.

Table 2 Summary of the characterization of 209/F_{1a} B-cell hybridomas

	Anti-TNP titre 3^n	IEF				IgM	Id^+	Presence of insert	mRNA		J_H probe No. of bands	*D* probe No. of *J*-bands	Possible protein	Phenotype DNA heavy-chain
	n	μ6	κ6	L	SDS	(μg ml^{-1})	(μg ml^{-1})	DNA	V_μ6	V_κ6	detected	detected		
Sp6	8	+	+	–	μ6, κ6	10	10	–	+	+	2	0	μ6κ6	*VDJ*$^+$, *VDJ*$^-$
1 D3-55	4	+	+	+	μ, κ	14	2	+	+	+	2	1	μ6κ6/κ	*VDJ*$^-$, *DJ*
2 E5-28	3	+	+	+	μ, κ	5	1	+	+	+	2	2	μ6κ6/κ	*DJ*, *DJ*
3 D10-29	2	+	+	+	μ, κ	4	0.7	+	+	+	2	2	μ6κ6/κ	*DJ*, *DJ*
4 C5-20	3	*	+	+	μ, κ, κ6	3.5	0.9	+	+	+	2	0	μ6κ6/μ, κ	*VDJ*$^+$, *VDJ*$^-$
5 H2-21	Nil	*	+	+	μ, κ, κ6	4.5	0.8	+	+	+	1	1	μ6κ6/κ	*DJ*
6 H1-28	4	+	+	+	μ, κ, κ6	2	0.8	+	+	+	1	1	μ6κ6/κ	*DJ*
7 E11-13	7	+	+	+	μ, κ	2.5	1.4	+	+	+	2	1	μ6κ6/κ	*DJ*, germ line
8 H5-33	Nil	*	+	+	μ, κ	10	1.5	+	+	+	2	1	μ6κ6/μ, κ	*VDJ*$^+$*DJ*
9 H5-29	1	Low		+	κ, (κ6)	0.04	0.08	+	+	+	1	1	(μ6κ6)/κ	*DJ*
10 C3-24	4	*	+	+	μ, κ, κ6	2	0.6	+	+	+	2	0	μ6κ6/μ, κ	*VDJ*$^+$/*VDJ*$^-$
11 D6-4	6	+	+	+	μ, κ	ND	ND	+	ND	ND	2	2	μ6κ6/κ	*DJ*/*DJ*
C5-20-22	6	As C5-20				12	6	+	+	+	2	ND	μ6κ6/μ, κ	
C5-20-16	3	+	–	+	μ, κ	7	<0.04	–	–	–	2	ND	μ, κ	
D3-55-19-60	5	As D3-55				30	11	+	ND	ND	2	ND	μ6κ6/κ	
D3-55-19-63	Nil	–	–	–	–	–	–	–	ND	ND	2	ND	Nil	
E11-34-46	8	As E11-13				30	10	+	ND	ND	2	ND	μ6κ6/κ	
E11-34-47	Nil	–	–	+	κ	–	–	–	ND	ND	2	ND	κ	

TNP/SRBC agglutination, IgM and idiotype (Id^+) titres were measured on supernatants of the indicated lines as described for Table 1. For IEF, + indicates a band or a series of bands co-migrating with κ6 or μ6 in the presence of an endogenously rearranged second light (L) chain; SDS pattern, presence of material running at the positions of the μ and κ is indicated; κ6 indicates the presence of a second light-chain band co-migrating with the Sp6 κ-chain. Possible phenotypes are written for proteins as μ6 and κ6 representing Sp6-like IgM, μ and κ representing additional endogenous chains. Among the rearrangements at the J_H locus, *V–D–J*$^-$ indicates a putatively inactive rearrangement. In H2-21, co-expression of two heavy chains is implied by the Id^+/TNP^- phenotype and the IEF pattern, but we were unable to reveal a second J_H-containing DNA band using seven restriction enzymes. In E11-13, using seven restriction enzymes, a J_H-containing DNA band co-migrating with the germ line J_H band was found. ND, not done. * Additional bands present.



209FlaxSp2/0

	LIGHT CHAIN GENES					HEAVY CHAIN GENES				
	1	2	3	4	5	1	2	3	4	5
V	142	197	148	203	811	1253	1235	1420	688	1959
C	2889	3592	2344	1068	773	485	435	485	459	523
V/Sp6	.17	.24	.18	.25	1.0	.63	.63	.72	.35	1.0
C/Sp6	3.7	4.6	3.0	1.4	1.0	.92	.83	.92	.88	1.0

Fig. 3 Northern blots on RNA from B-cell hybridomas. Total cytoplasmic RNA (30 μg) was separated on agarose gels as described previously[31]. The filters were probed with ^{32}P-labelled pBR322 plasmid DNA ($2–8 \times 10^8$ c.p.m. μg^{-1}) containing the entire $V_\mu6$ (*a*) or $V_\kappa6$ (*b*) region. Purified fragments containing $V_\kappa6$, C_κ and $V_\mu6$ coding sequences and pABμ-1 containing C_μ (ref. 32) were used to derive the quantitative estimates listed below. Numbers 1–5 correspond to lines D3-55, E5-28, D10-29, C5-20 and Sp6, respectively.

Table 2 also shows the deduced organization of their heavy-chain loci, as well as the putative expression of endogenous heavy and light chains. A V-D-J^--rearrangement is defective for μ protein production. This is shown in D3 by the failure of μ-chain production in its subclone (which had lost the pR-HL_{TNP} DNA) (Table 2). The V-D-J^--alleles of C5-20 and C3-24 were assumed to be defective, as no evidence of a third μ-chain was found by IEF-PAGE.

Discussion

Our analysis shows that not only μ (11) and κ (9) genes separately but also both together can be introduced into the mouse germ line and expressed in an orderly fashion in B cells (IgM membrane deposition, secreted pentameric and functional IgM in serum). A third gene, *neo*, encoding neomycin resistance and under SV40 promoter/enhancer control, does not protect hybrid cells from the toxicity of the neomycin analogue G418, nor do we find *neo*-specific cytoplasmic RNA of the correct size (data not shown). It remains to be seen whether this gene is still intact in transgenic mice.

In normal mice, a cell never expresses more than one heavy-chain product or more than one light-chain product[4,16]. This allelic exclusion is thought to be necessary for effective clonal selection. Expression of the Sp6 light chain in the B hybridomas was at about one-tenth the level of the co-expressed light chain, and no inhibition of intrinsic light-chain rearrangement was observed. This may be possible because an excess of transgenic μ-chain mRNA and protein occurs in the same cell, thus mimicking the situation in normal B-cell development, where μ appear first[17].

Ritchie *et al.*[18] found that light-chain rearrangement is inhibited when both endogenous μ and the transgenic light chain are synthesized in quantities comparable with those in hybridomas derived from normal mice. Our results, on the other hand, indicate that μ-light-chain molecules in low concentrations with an excess of free heavy chains do not have a measurable effect on the process of light-chain gene rearrangement. Thus, the existence of μ light-chain molecules *per se* is not important in the regulation of the V_L-J_L joining mechanism. It does not contradict models in which the amount of light chain is essential in inhibiting rearrangement[19].

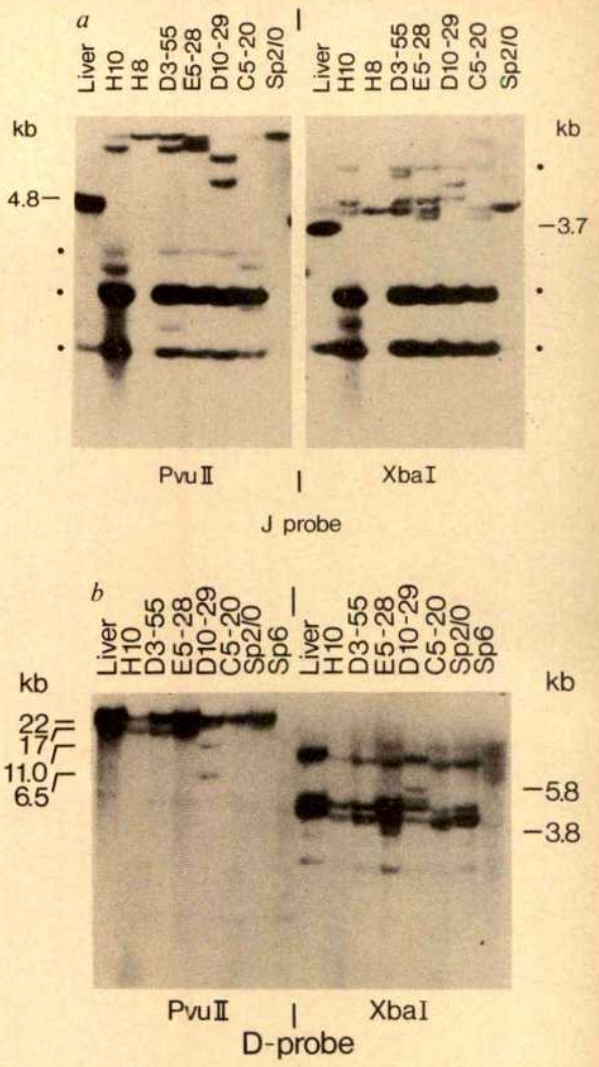


Fig. 4 Joining configuration at the J_H-locus analysed by Southern blotting. *a*, *J* probe; *b*, *D* probe. DNA (15 μg) from the indicated B-cell hybridomas was separated on 1% agarose gels and blotted onto nitrocellulose filters, which were then hybridized to nick-translated, purified DNA probes. The *Bam*HI/*Eco*RI fragment containing the J_H3/J_H4 segment was purified from a pBR322 plasmid containing all four mouse J_H segments (gift of M. Steinmetz). The J_H probe was labelled to 5×10^8 c.p.m. μg^{-1} and was used in the blot shown in *a*. The *Pst*I/*Pst*I fragment containing the 5′ *D*Sp2 segment was purified from the plasmid p4-7-5 (ref. 4; gift of M. Reth). The 5′ *D*Sp2 probe was labelled to 6×10^6 c.p.m. μg^{-1} and was used in the blot in *b*. Cutting the DNA of H10, D3-55 and E5-28 with both *Pvu*II or *Xba*I restriction enzymes reveals a common band hybridizing to the J_H probe, also visualized using the 5′ *D*Sp2 probe (*Pvu*II, 17 kb). This band is hidden in the series of bands seen between 3.8 and 5.8 kb after *Xba*I digestion. The 3.8-kb band of E5-28 DNA (*Xba*I) therefore reflects the DNA coming from the 22-kb (*Pvu*II) band, which is difficult to reproduce in the photograph. All DNA samples were also tested with DFl-16, representative of a second D′-family[4] (data not shown).

Table 3 Comparison of the rearrangement of J_H alleles of various sources

	V-D-J^+	V-D-J^-	D-J (%)	Germ line	Total
Hybrids (this paper)	3	3	12(63)	1	19
Abelson* fetal liver	1	7	18(69)	0	26
Abelson* bone marrow	9	15	7(23)	0	31
Myeloma* silent allele	—	20	12(38)	0	32

* From ref. 4.

At the heavy-chain locus, co-expression of μ-chains occurs in the C5-20 line; the variant C5-20-16, which has lost pR-HL_{TNP}, still synthesizes μ-chain (Fig. 2). Co-expression is also probable in lines H5-33, C3-24 and the γ-chain-expressing lines 46-43 and 44 (Fig. 2, Table 2). The amounts of μ6 produced are low in at least three of the four lines (H5-33, C3-24 and 46-44; Fig. 2). The amounts of μ6 produced by C5-20 could, however, be approximately equivalent to the amount of the endogenously produced μ-chain). In the λ-expressing 18-7 line, μ6 and endogenous μ are made in about equal amounts (data not shown). Nevertheless, of 11 hybrids analysed in detail, 8 express μ6 alone, and as 5 of these retain both heavy-chain loci, chromosome loss in the hybridoma could not account for loss of endogenous μ-chain production (Table 2). By isolation of Sp6/IgM-negative variants of the D3 and E11 lines we have confirmed that there is no endogenous μ production (Table 2).

One of the 19 heavy-chain loci analysed here is arrested in the germline configuration (E11-13), 12 remain in the immature *D-J* configuration and 6 complete the entire *V-D-J* coding unit, even though only 3 of them actually produce μ-chains.

Compared with Abelson murine leukaemia virus-transformed bone marrow or myeloma B-cell lines, the transgenic mouse has a high frequency of cells in the immature joining configuration (Table 3). There are two possible explanations: first, B cells that express Sp6 IgM early in their development might be stimulated by LPS and thereby hybridize earlier than normal B cells. On fusion, the rearrangement machinery is inactivated so that their DNA is frozen in the immature configuration. If, however, light-chain follows heavy-chain gene rearrangement[20-22], this possibility seems unlikely, as all hybrids express an endogenous light chain. Second, early expression of Sp6 IgM might slow down directly the process of J_H rearrangement. As most lines have made the D-J_H rearrangement, this step must be less vulnerable to inhibition, either because D to J joining precedes competence of a B cell for μ expression or because immunoglobulin chains do not greatly affect this step.

Our results agree with models postulating negative feedback control mechanisms of μ-chain on the process of J_H rearrangement[4]. They do not support a mechanism in which allelic exclusion at the heavy-chain locus is explained by elimination of B cells having two μ-producing rearrangements[19].

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1. Tonegawa, S. *Nature* **302**, 575-581 (1983).
2. Adams, J. M. *et al. Immun. Rev.* **59**, 5-32 (1981).
3. Honjo, T. *et al. Immun. Rev.* **59**, 33-67 (1981).
4. Alt, W. F. *et al. EMBO J.* **3**, 1209-1219 (1984).
5. Constantini, G. & Lacy, E. *Nature* **294**, 92-94 (1981).
6. Wagner, E. F., Stewart, T. A. & Mintz, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5016-5020 (1981).
7. Palmiter, R. D., Chen, H. Y. & Brinster, R. L. *Cell* **29**, 701-710 (1982).
8. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. *Science* **222**, 809-814 (1983).
9. Brinster, R. L. *et al. Nature* **306**, 332-336 (1983).
10. Storb, U., O'Brien, R. L., McMullen, M. D., Gollahon, K. A. Brinster, R. L. *Nature* **310**, 238-241 (1984).
11. Grosschedl, R., Weaver, D., Baltimore, D. & Constantini, F. *Cell* **38**, 647-658 (1984).
12. Köhler, G. & Milstein, C. *Eur. J. Immun.* **6**, 511-519 (1976).
13. Ochi, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 6351-6355 (1983).
14. Shulman, M., Wilde, D. C. & Köhler, G. *Nature* **276**, 269-270 (1978).
15. Köhler, G., Potash, M. J., Lehrach, H. & Shulman, M. *EMBO J.* **1**, 555-563 (1982).
16. Alt, F. W., Enea, V., Bothwell, A. L. M. & Baltimore, D. *Cell* **21**, 1-12 (1980).
17. Kincade, P. W. *Adv. Immun.* **31**, 177-245 (1981).
18. Ritchie, K. A., Brinster, R. L. & Storb, U. *Nature* **312**, 517-520 (1984).
19. Wabl, M. & Steinberg, C. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6976-6978 (1982).
20. Alt, F. W., Rosenberg, N., Lewis, S., Thomas, E. & Baltimore, D. *Cell* **27**, 381-390 (1981).
21. Perry, R. P., Kelley, D. E., Coleclough, C. & Kearney, J. K. *Proc. natn. Acad. Sci. U.S.A.* **78**, 247-251 (1981).
22. Korsmeyer, S. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 7096-7100 (1981).
23. Leptin, M. *et al. Eur. J. Immun.* **14**, 534-542 (1984).
24. Yelton, D. E., Desaymard, C. & Scharff, M. D. *Hybridoma* **1**, 5-11 (1981).
25. Weiss, S., Lehmann, K. & Cohn, M. *Hybridoma* **2**, 49-54 (1983).
26. Southern, E. M. & Berg, P. *J. molec. appl. Gen.* **1**, 327-341 (1982).
27. Rusconi, S. in *The Impact of Gene Transfer Techniques in Eucaryotic Cell Biology* (eds Schell, J. & Starlinger, P.) (Springer, New York, in the press).
28. Brinster, R. L. *et al. Cell* **27**, 223-231 (1981).
29. Kafatos, F. C., Jones, C. W. & Efstratiadis, A. *Nucleic Acids Res.* **7**, 1541-1552 (1979).
30. Rusconi, S. *Cell* (submitted).
31. McMaster, G. K. & Carmichael, G. G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4835-4838 (1977).
32. Alt, F. W. *et al. Cell* **20**, 293-301 (1980).

LETTERS TO NATURE

Gravitational radiation from a solid-crust neutron star

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The measured period derivative[1-3] for the millisecond pulsar PSR1937+21[4] is $\dot{P} \simeq 1.05 \times 10^{-19}$, whereas the present upper limit[5] for PSR1953+29[6] is $\dot{P} < 4.2 \times 10^{-17}$. For electromagnetic braking, the combination of rapid rotation with such small period derivatives requires a small ($\sim 10^8$ G) magnetic field. A natural association between rapid rotation and small magnetic field is provided by accretion spin-up scenarios[7-9]. Here we address the analogous problem for gravitational radiation. Balancing gravitational radiation power against the rate of rotational energy loss, we find that if the effective triaxiality $\varepsilon_{\rm eff}$ exceeds $\varepsilon_{\rm max} \sim 10^{-9}$, gravitational radiation alone would spin a millisecond pulsar down at a faster rate than the observed $\dot{P}$. We show that $\varepsilon_{\rm eff} \ll \varepsilon_{\rm max}$, so that gravitational radiation is not likely to play a major role in the evolution of millisecond pulsars even in the absence of damping. Damping times for precession of the solid crust are estimated to be $< 2 \times 10^3$ yr.

The energy loss rate due to gravitational radiation is

$$I\Omega\dot{\Omega} = \frac{32}{5}\frac{G}{c^5} I^2 \varepsilon_{\rm eff}^2 \Omega^6 \qquad (1)$$

where I is the moment of inertia, Ω the angular velocity and $\varepsilon_{\rm eff}$ the effective triaxiality. With P and $\dot{P}$ values similar to those for PSR1937+21,

$$\varepsilon_{\rm max} = 1.9 \times 10^{-9} \left(\frac{\dot{P}_{-19}}{I_{45}}\right)^{1/2} P(\rm ms)^{3/2} \qquad (2)$$

which seems to impose a constraint on millisecond pulsars as their rotational oblateness, $\varepsilon_\Omega \equiv I\Omega^2/4A$, is $\sim 10^7$ times larger (see Table 1).

The moment of inertia tensor of a neutron star with a solid crust is

$$\bar{I} = I_0 \left[\left(1 - \frac{\varepsilon_\Omega}{2} - \frac{b\varepsilon_0}{2}\right)\bar{e} + \frac{3}{2}\varepsilon_\Omega \hat{n}_\Omega \hat{n}_\Omega + \frac{3}{2} b\varepsilon_0 \hat{n}_0 \hat{n}_0 \right] \qquad (3)$$

where I_0 is the moment of inertia in the spherical case (no rotation), ε_Ω the 'rotational oblateness', ε_0 the 'reference oblateness' of the solid crust, b is a coefficient describing its rigidity, $\hat{n}_0$ and $\hat{n}_\Omega$ are unit vectors along the reference axis and the instantaneous angular velocity vector $\vec{\Omega}$, and $\bar{e}$ is the unit tensor[10]. Note that the triaxiality of $\bar{I}$ stems from b, the rigidity of the solid. The corresponding fluid star ($b = 0$) would have

an axially symmetric equilibrium shape. (Wagoner[11] finds that non-axially symmetric perturbations of a fluid neutron star can grow only at angular velocities 1.5–2 times faster than that of PSR1937+21. Friedman[12] finds that non-axial perturbations are stable at $P \simeq 1.5$ ms for some equations of state; this result, however, rests on the assumption that the viscosity is that appropriate for a temperature $T \sim 10^9$ K that is far too high for the quite-old millisecond pulsars. We thus conclude that the underlying fluid equilibrium shape is axially symmetric.)

The $\hat{n}_\Omega \hat{n}_\Omega$ term in $\vec{I}$ acts like the unit tensor $\vec{e}$ on the $\vec{\Omega}$ vector. The kinetic energy, T, then has the same form as that of a symmetric top with symmetry axis $\hat{n}_0$

$$T = \frac{1}{2} I_0 \left[(1 + \varepsilon_\Omega + b\varepsilon_0)\Omega_3^2 + \left(1 + \varepsilon_\Omega - \frac{b\varepsilon_0}{2}\right)\Omega_1^2 + \left(1 + \varepsilon_\Omega - \frac{b\varepsilon_0}{2}\right)\Omega_2^2 \right] \quad (4)$$

where Ω_3 is the component of $\vec{\Omega}$ along $\hat{n}_0$. Then $\hat{n}_0$, $\vec{\Omega}$ and the angular momentum $\vec{L}$ all lie in a plane, which precesses about the fixed $\vec{L}$ axis, with a precession frequency in the non-rotating frame given by[13]

$$\Omega_{pr} = \frac{L}{I_1} = \frac{L}{I_0}\left(1 - \varepsilon_\Omega + \frac{b\varepsilon_0}{2}\right) \simeq \Omega\left(1 + \frac{3b\varepsilon_0}{2}\cos^2\theta_0\right) \quad (5)$$

where θ_0 is the angle between $\vec{\Omega}$ and $\hat{n}_0$. We have a triaxial body, which acts dynamically like a symmetric top because the rotationally induced contribution to the non-sphericity of $\vec{I}$ has $\vec{\Omega}$ itself as a symmetry axis. To diagonalize $\vec{I}$, a rotation in the $(\vec{L},\vec{\Omega})$ plane is required. As the two independent vectors $\hat{n}_0$ and $\hat{n}_\Omega$ lie in this plane, so do two principal axes, $\hat{n}_1$ and $\hat{m}_1$. The $(\vec{L},\vec{\Omega})$ plane is a symmetry plane of the triaxial neutron star, which therefore precesses about $\vec{L}$ at the frequency Ω_{pr}, so that this is also the observed pulsar frequency for a pulsar beam fixed in the body frame.

The gravitational radiation power in a frequency ω is[14]

$$P(\omega) = \frac{2G\omega^6}{5c^5}\{D_{ij}^*(\omega)D_{ij}(\omega) - \tfrac{1}{3}|D_{ii}(\omega)|^2\} \quad (6)$$

where

$$D_{ij}(t) = \sum_{\omega>0} \exp(-i\omega t)D_{ij}(\omega) + \text{complex conjugate} \quad (7)$$

is the moment

$$D_{ij}(t) = M(t)_{ia}M(t)_{jb}\int d^3x' x'_a x'_b \rho(x') = M(t)_{ia}M(t)_{jb}\left(\frac{\text{tr}\, I}{2}\delta_{ab} - I_{ab}\right) \quad (8)$$

Here $\rho(x')$ is the mass density, $\vec{x}'$ is the position vector in the body frame defined by the principal axes and $M(t)$ is the instantaneous transformation matrix from the body frame $\hat{m}_1(t)$, $\hat{n}_1(t)$, $\hat{m}_1(t)\times\hat{n}_1(t)$ to a fixed frame, whose $\hat{x}_3$ axis we choose to be parallel to $\vec{L}$ (see Fig. 1). Gravitational radiation comes out in the frequencies Ω_{pr} and $2\Omega_{pr}$. Diagonalizing $\vec{I}$ to find $\hat{m}_1(t)$, $\hat{n}_1(t)$, $\hat{m}_1(t)\times\hat{n}_1(t)$ and thus the matrix $M(t)$, equations (3)–(8) yield the output power, to lowest order in $b\varepsilon_0$ and $b\varepsilon_0/\varepsilon_\Omega$

$$P(2\Omega_{pr}) \simeq \frac{72}{5}\frac{GI_0^5}{c^5}\Omega_{pr}^6 b^2\varepsilon_0^2 \sin^4\theta_0 \quad (9)$$

$$P(\Omega_{pr}) \simeq \frac{9}{10}\frac{GI_0^2}{c^5}\Omega_{pr}^6(1-\tfrac{3}{2}\varepsilon_\Omega)^2 b^2\varepsilon_0^2\cos^2\theta_0\sin^2\theta_0 \quad (10)$$

Comparing with equation (1)

$$\varepsilon_{eff}^2 = (\tfrac{3}{2}b\varepsilon_0\sin^2\theta_0)^2 + (\tfrac{3}{8}b\varepsilon_0(1-\tfrac{3}{2}\varepsilon_\Omega)\cos\theta_0\sin\theta_0)^2 \quad (11)$$

These are essentially the well-known results for a precessing axially symmetric body[15]. The rotational oblateness $\varepsilon_\Omega \hat{n}_\Omega \hat{n}_\Omega$ contributes to higher order corrections.

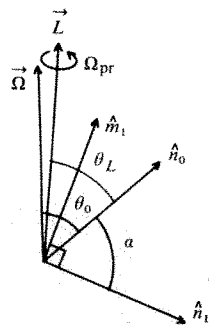


Fig. 1 The reference plane containing $\vec{L}$, $\vec{\Omega}$, $\hat{n}_0$ and the principal axes $\hat{m}_1$ and $\hat{n}_1$ of the triaxial solid-crust neutron star. This plane precesses at the rate Ω_{pr} around the angular momentum axis $\vec{L}$. We have $\theta_L = \theta_0(1 - O(b\varepsilon_0))$ and $\alpha = \pi/2 - \theta_0(1 - O(b\varepsilon_0/\varepsilon_\Omega))$.

We now consider the values of b, ε_0 and θ_0 for a millisecond pulsar that has been spun up by accretion and has been spinning down as a pulsar since the end of the accretion phase[7].

(1) The rigidity, b. A solid with oblateness, ε, and reference oblateness, ε_0, has an elastic energy $B(\varepsilon - \varepsilon_0)^2$; the oblateness increases the gravitational potential energy by an amount $A\varepsilon^2$ above the gravitational energy of a spherical star of the same mass. The coefficient b in equation (3) is defined by $b \equiv B/(A+B)$ and Pandharipande, Pines and Smith[16] have calculated A and B for a variety of neutron star models. Typical values imply $b \sim 10^{-5}$.

(2) The reference oblateness ε_0. ε_Ω is the equilibrium oblateness of a fluid body rotating at angular velocity Ω, $\varepsilon_\Omega \equiv I_0\Omega^2/4A$. The reference oblateness ε_0 is defined as the oblateness of the star at the time of formation of its solid crust, modified subsequently according to its seismic history, through plastic flow or starquakes[17,18]. If we make the conservative assumption that a neutron star spun up by accretion has, at the time of crust solidification, a period of ~ 1 s, the initial value of the reference oblateness is $\varepsilon_0(0) \simeq I_0\Omega_0^2/4A \sim 10^{-6}$. Neglecting plastic flow associated with the logarithmic creep of dislocations in the crust[18], ε_0 will change only through starquakes, which take place when $|\varepsilon_\Omega - \varepsilon_0| \simeq \theta_m$, where θ_m, the critical strain angle of the solid crust, is $\sim 10^{-4}$–10^{-5} for typical terrestrial metals, whereas for a pure Coulomb lattice, which may be a better approximation to the neutron star crust[19], it is $\sim 10^{-1}$–10^{-2}. We consider various possibilities for the evolution of ε_0: (i) $\theta_m \sim 10^{-4}$. ε_0 follows the accretion spin-up and the subsequent pulsar spin-down by a series of quakes, starting at a time when $\varepsilon_\Omega \sim 10^{-4}$, $\varepsilon_0 \sim 10^{-6}$, to its present value $\varepsilon_0 \simeq \varepsilon_\Omega \simeq 10^{-1}$(PSR1937+21) within $\theta_m \sim 10^{-4}$ of ε_Ω. Even if the reference oblateness, ε_0, were also affected by solidification of accreted material, our above conclusion would not change, as the smallness of the critical angle, θ_m, and the consequent large number of quakes during the $\sim 10^8$-yr accretion era and the subsequent and comparably long pulsar era will bring the final ε_0 close to ε_Ω. (ii) $\theta_m \sim 10^{-1}$. The reference oblateness, ε_0, may keep its initial value of $\sim 10^{-6}$ through the entire spin-up era, without reaching the condition $|\varepsilon_\Omega - \varepsilon_0| \simeq \theta_m$ for quakes. The subsequent decrease of ε_Ω, during spin-down as a pulsar, will decrease the difference $\varepsilon_\Omega - \varepsilon_0$; the present reference oblateness is $\varepsilon_0 \simeq \varepsilon_0(0) \sim 10^{-6}$. For large $\theta_m \sim 10^{-1}$, one may consider the possibility that ε_0 grows by solidification of accreted material in a way that does not follow the evolution of ε_Ω. However, the magnetic field that channels the accreted material plays a negligible part in the equilibrium energy balance, on the star's surface in comparison with rotation; $(B^2/8\pi)(4\pi R^3/3I_0\Omega^2)$ is $\sim 4\times 10^{-6}$, for $B = 10^{12}$ G and $P = 1$s, and $\sim 10^{-19}$, for $B = 10^8$ G and $P = 1.5$ ms. Furthermore, although the total amount of matter accreted is $\sim 0.1 M_\odot$, it is unlikely that this can adhere, in a metastable way, to the magnetic-field-accretion geometry and solidify to change ε_0 on timescales as long as the accretion time of $\sim 10^8$ yr; the temperature of the accreted matter is in the X-ray range through this era, so

Table 1 Values of $\varepsilon_{\rm eff}$ and ε_Ω for the millisecond and binary pulsars

Pulsar	1937+21	1953+29	1913+16	0820+02	0655+64	'Ordinary'
P(ms)	1.6	6.1	59	865	196	200
$\dot{P}(10^{-19}\,{\rm s\,s^{-1}})$	1.05*	7.8†	86	10^3	<10‡	10^5
$\varepsilon_{\rm eff}(I_{45}^{-1/2})$	3.8×10^{-9}	8×10^{-8}	8×10^{-6}	1.5×10^{-3}	$<1.6\times10^{-5}$	1.7×10^{-3}
$\varepsilon_\Omega(I_{45}/A_{53})$§	4.1×10^{-2}	2.7×10^{-3}	2.8×10^{-5}	1.3×10^{-7}	2.5×10^{-6}	2.5×10^{-6}

The last column lists $\varepsilon_{\rm eff}$ and ε_Ω for P and $\dot{P}$ values typical of an ordinary pulsar.
* Ref. 3.
† Scaling as $P^{4/3}$ from the observed value for PSR1937+21. The present observational upper limit is $\dot{P}<4.2\times10^{-17}$.
‡ Present upper limit (A. G. Lyne, personal communication).
§ A is the coefficient in the additional gravitational potential energy, $A\varepsilon^2$, of the star due to an oblateness ε with typical values $\sim10^{53}$ erg (ref. 16).

diffusion and plastic flow timescales are likely to be much shorter than 10^8 yr.

(3) The angle θ_0 between $\hat{\boldsymbol{n}}_0$ and $\hat{\boldsymbol{n}}_\Omega$. This can also change through quakes. Pines and Shaham[20] have shown that the critical value of θ_0 for such a 'θ-quake' is reached when $3\varepsilon_\Omega\varepsilon_0\theta_0^2\simeq\theta_{\rm m}^2$. The different possibilities are: (i) for $\theta_{\rm m}\sim10^{-4}$ and $\varepsilon_0\simeq\varepsilon_\Omega\sim10^{-1}$, the critical value of θ_0 is $\sim10^{-3}$. Thus, a crust with a small critical angle cannot sustain any misalignment between $\hat{\boldsymbol{n}}_0$ and $\hat{\boldsymbol{n}}_\Omega$ greater than $\sim10^{-3}$ rad. $\theta_0\simeq0$ at initial solidification and cannot exceed $\sim10^{-3}$ through the star's history. (ii) For $\theta_{\rm m}\sim10^{-1}$, $\varepsilon_\Omega\simeq10^{-1}$ and $\varepsilon_0\simeq10^{-6}$, no bound is imposed on θ_0. Starting at $\theta_0\simeq0$ at solidification, θ_0 could in principle grow arbitrarily, without inducing a 'θ-quake' in such a strong crust; but one needs an evolutionary reason for a secular increase of θ_0 and the only candidate, solidification of accreted material persisting through the 10^8-yr accretion phase, is ruled out by the considerations given above in connection with the related question of the evolution of ε_0 for a $\theta_{\rm m}\sim0.1$ crust.

(4) The effect of pinned superfluidity. The presence of a pinned superfluid will not alter the situation. Shaham[21] has investigated the effect of the pinned superfluid on the rotational dynamics of a neutron star. Pinning leads to an angular momentum $\vec{L}_{\rm f}$ that is constant in the rotating frame of the star. Shaham shows that the precession frequency in the rotating frame is of the order of $L_{\rm f}/I_0$. We find

$$\frac{L_{\rm f}}{I_0}\simeq\frac{I_{\rm p}}{I_0}(\Omega+\omega_{\rm cr})\simeq\frac{I_{\rm p}}{I_0}\Omega\simeq10^{-2}\Omega \qquad (12)$$

where $I_{\rm p}\simeq10^{-2}I_0$ is the moment of inertia of the pinned superfluid and $\omega_{\rm cr}\leqslant1$ rad s^{-1} is a critical frequency for unpinning[22,23]. The corresponding frequency in the non-rotating frame is given by

$$\Omega_{\rm pr}=\Omega\left(1+{\rm O}\left(\frac{L_{\rm f}}{I_0\Omega}\right)+\frac{3}{2}b\varepsilon_0\cos^2\theta_0\right)=\Omega(1+{\rm O}(10^{-2}))\simeq\Omega \qquad (13)$$

For the values of $L_{\rm f}$ given by neutron star pinning parameters, the gravitational radiation emitted by the neutron star deviates little from that in the absence of pinning. As $\Omega_{\rm s}$, the rotation rate of the pinned superfluid, exceeds Ω by at most the critical frequency, $\omega_{\rm cr}$, that is, $|\vec{\Omega}_{\rm s}-\vec{\Omega}|<\omega_{\rm cr}\leqslant1$ rad s^{-1}, the angle ϕ between $\vec{L}_{\rm f}$ and $\vec{\Omega}$ will be small, that is, $\phi<\omega_{\rm cr}/\Omega\sim10^{-3}$. The angle $\theta_0-\theta_L$ between $\vec{\Omega}$ and $\vec{L}$ is also small. Using $\vec{L}=\vec{I}\cdot\vec{\Omega}+\vec{L}_{\rm f}$ we find $\theta_0-\theta_L\simeq(I_{\rm p}/I_0)\phi<(I_{\rm p}/I_0)(\omega_{\rm cr}/\Omega)\sim10^{-5}$. The deviations of $\vec{\Omega}$ and $\vec{L}_{\rm f}$ from the direction of $\vec{L}$ are very limited. A comparison with our calculation for gravitational radiation in the absence of pinning shows that the only effect on $M(t)$ is to replace $O(b\varepsilon_0)$ expressions for the angle $(\theta_L-\theta_0)$ with another negligible quantity, as $\theta_L-\theta_0$ is now $(I_{\rm p}/I_0)(\omega_{\rm cr}/\Omega)\sim10^{-5}$. The lowest-order results for the gravitational radiation output are not affected, nor are our evaluations of the effective triaxiality under different assumptions for the strength of the crust solid.

(5) The expected value of $\varepsilon_{\rm eff}$. We thus conclude that the effective triaxiality, $\varepsilon_{\rm eff}$, in equation (11) is: (i) $\varepsilon_{\rm eff}\simeq\frac{3}{8}b\varepsilon_0\theta_0\simeq\frac{3}{8}\times10^{-5}\times10^{-1}\times10^{-3}\simeq4\times10^{-10}$, if $\theta_{\rm m}\sim10^{-4}$; (ii) $\varepsilon_{\rm eff}\simeq b\varepsilon_0\simeq10^{-5}\times10^{-6}\simeq10^{-11}$, if $\theta_{\rm m}\sim10^{-1}$.

(6) Intrinsic triaxiality. Intrinsic triaxiality of the solid reference figure adds a term $\eta(\hat{\boldsymbol{l}}_0\hat{\boldsymbol{l}}_0-\hat{\boldsymbol{m}}_0\hat{\boldsymbol{m}}_0)$ to $\vec{\boldsymbol{I}}$ in equation (3), where η is the 'reference triaxiality', and $\hat{\boldsymbol{l}}_0$, $\hat{\boldsymbol{m}}_0$, $\hat{\boldsymbol{n}}_0$ form an orthogonal frame. It follows that $\varepsilon_{\rm eff}$ acquires a term of the order of $b\eta$, and that $\eta\leqslant\theta_{\rm m}$. Thus, $b\eta\leqslant10^{-5}\times10^{-4}\simeq10^{-9}$, if $\theta_{\rm m}\sim10^{-4}$. In the 'no quake' case, $\theta_{\rm m}\sim10^{-1}$, η retains its initial value, $\eta=\eta(0)\leqslant\varepsilon_0(0)\sim10^{-6}$, and one finds $b\eta\leqslant10^{-5}\times10^{-6}\simeq10^{-11}$, if $\theta_{\rm m}\sim10^{-1}$. Both limits are less than the observational limit $\varepsilon_{\rm max}$.

In conclusion, our results in parts (5) and (6) above show that gravitational radiation poses no problem for the observed $\dot{P}$ values of millisecond pulsars. A consideration of damping of the solid crust's precession through its coupling to the superfluid neutron star core strengthens this conclusion. The damping time $\tau_{\rm W}$ is[24,25]

$$\tau_{\rm W}\simeq\frac{\Omega}{\Omega'_{\rm pr}}\tau\simeq(\tfrac{1}{2}b\varepsilon_0)^{-1}\tau \qquad (14)$$

where $\Omega'_{\rm pr}$ is the precession frequency in the body frame and τ is the crust-core coupling time. A recent investigation[26] of the interaction of quantized vortices in the core neutron superfluid with electrons and protons yields a coupling time $\tau_{\rm v}\simeq20P({\rm s})$. The dynamical coupling time τ for the neutron superfluid is then $\tau({\rm s})\equiv\tau_{\rm v}/x\simeq400P({\rm s})$, where x, the ratio of the number of protons to that of neutrons, is typically $\sim5\%$. From equation (14) and the above value of τ, we obtain a damping time $\tau_{\rm W}({\rm yr})\leqslant1{,}300$ yr for PSR1937+21, using $b\varepsilon_0\sim10^{-11}$, whereas with pinning, $\Omega/\Omega'_{\rm pr}\sim100$ and $\tau_{\rm w}\simeq1$ min.

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1. Ashworth, M., Lyne, A. G. & Smith, F. G. *Nature* **301**, 313-314 (1983).
2. Backer, D. C., Kulkarni, R. S. & Taylor, J. H. *Nature* **301**, 314-315 (1983).
3. Backer, D. C. in *Proc. Workshop on Millisecond Pulsars* (eds Reynolds, S. & Stinebring, D.) 3-8 (NRAO, Greenbank, West Virginia, 1984).
4. Backer, D. C., Kulkarni, R. S., Heiles, C., Davis, M. M. & Goss, W. M. *Nature* **300**, 615-618 (1982).
5. Boriakoff, V., Buccheri, R., Fauci, F., Turner, K. & Davis, M. in *Proc. Workshop on Millisecond Pulsars* (eds Reynolds, S. & Stinebring, D.) 24-29 (NRAO, Greenbank, West Virginia, 1984).
6. Boriakoff, V., Buccheri, R. & Fauci, F. *Nature* **304**, 417-419 (1983).
7. Alpar, M. A., Cheng, A. F., Ruderman, M. A. & Shaham, J. *Nature* **300**, 728-730 (1982).
8. Radhakrishnan, V. & Srinivasan, G. *Curr. Sci.* **51**, 1096-1099 (1982).
9. Fabian, A. C., Pringle, J. E., Verbunt, F. & Wade, K. A. *Nature* **301**, 222-223 (1983).
10. Pines, D. & Shaham, J. *Phys. Earth planet. Inter.* **6**, 103-115 (1972).
11. Wagoner, R. *Astrophys J.* **278**, 345-348 (1984).
12. Friedman, J. L. *Phys. Rev. Lett.* **51**, 11-14 (1983).
13. Landau, L. D. & Lifshitz, E. M. *Mechanics* Ch. 6 (Pergamon, Oxford, 1976).
14. Weinberg, S. *Gravitation and Cosmology* Ch. 10 (Wiley, New York, 1972).
15. Shapiro, S. L. & Teukolsky, S. A. *Black Holes, White Dwarfs and Neutron Stars* Ch. 16 (Wiley-Interscience, New York, 1983).
16. Pandharipande, V. R., Pines, D. & Smith, R. A. *Astrophys. J.* **208**, 550-566 (1976).
17. Ruderman, M. A. *Nature phys. Sci.* **223**, 597-598 (1969).
18. Baym, G. & Pines, D. *Ann. Phys.* **66**, 816-835 (1971).
19. Smoluchowski, R. *Phys. Rev. Lett.* **24**, 923-925 (1970).
20. Pines, D. & Shaham, J. *Nature phys. Sci.* **235**, 43-49 (1972).
21. Shaham, J. *Astrophys. J.* **214**, 251-260 (1977).
22. Alpar, M. A., Anderson, P. W., Pines, D. & Shaham, J. *Astrophys. J.* **276**, 325-334 (1984).
23. Pines, D. & Alpar, M. A. *Proc. Workshop on Millisecond Pulsars* (eds Reynolds, S. & Stinebring, D.) 161-169 (NRAO, Greenbank, 1984).
24. Bondi, H. & Gold, T. *Mon. Not. R. astr. Soc.* **115**, 41-46 (1955).
25. Pines, D. & Shaham, J. *Nature* **248**, 483-486 (1974).
26. Alpar, M. A., Langer, S. A. & Sauls, J. A. *Astrophys. J.* **282**, 533-541 (1984).

Hubble's constant and exploding carbon–oxygen white dwarf models for Type I supernovae

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The immediate progenitor of a Type I supernova (SN I) is thought to be a mass-accreting carbon–oxygen (C–O) white dwarf in a binary system. When the mass of the white dwarf approaches the Chandrasekhar mass (1.4 $M_\odot$) the C–O nuclear fuel ignites, part of the star is incinerated to radioactive ^{56}Ni, and the thermonuclear energy completely disrupts the star. The optical luminosity results from the trapping and thermalization of the γ rays and positrons emitted by the decay of ^{56}Ni through ^{56}Co to stable ^{56}Fe. The amount of ^{56}Ni synthesized, M_{Ni}, and the corresponding peak luminosity, L_{max}, can be used with the observed Hubble diagram for SN I to determine the value of Hubble's constant, H_0. We argue here that if this model is correct, M_{Ni} is in the range 0.4–1.4 $M_\odot$, the best estimate being 0.6 $M_\odot$, and that H_0 is in the range 39–73 km s^{-1} Mpc^{-1} with a best estimate of 59 km s^{-1} Mpc^{-1}. This line of reasoning does not require knowledge of the temperature of the supernova and, therefore, is not subject to the uncertainties associated with attempts to determine supernova luminosities and distances by the Baade method[1]. It relies on the physical correctness of the model, which is subject to independent tests.

For SN I, analytical solutions[2] for the light curves predict that at the time of maximum bolometric luminosity, L_{max} is equal to the instantaneous decay luminosity of the ^{56}Ni and ^{56}Co independent of the opacity and the precise form of the deposition function. Numerical calculations confirm the insensitivity of the peak bolometric luminosity to opacity[3] and show that the radiated power is equal to the rate of radioactive deposition at bolometric maximum to within 15%. The time between explosion and maximum luminosity in the B-band is observed to be 15 ± 2 days (ref. 4), which implies that maximum bolometric luminosity occurs at 17 ± 2 days (ref. 3). It follows that $L_{max} = 2.2 \pm 0.2 \times 10^{43}\ M_{Ni}$ erg s^{-1} $M_\odot^{-1}$. This is a significant simplification; at times away from maximum luminosity, the luminosity is affected by the opacity of the matter and hence its composition, thus introducing additional uncertainty.

For an exploding white dwarf, a rigorous and extreme upper limit on M_{Ni} is 1.4 $M_\odot$. Optical spectroscopy of extragalactic SN I[5] and X-ray spectroscopy of remnants of SN I in our Galaxy (C. Sarazin, personal communication) show that the ejected matter contains synthesized elements such as silicon and sulphur, and unburned carbon (presupposing the model) and oxygen in addition to iron-peak elements so the entire 1.4 $M_\odot$ cannot be ^{56}Ni.

A lower limit on M_{Ni} follows from the observed kinetic energy and velocities. To an excellent approximation the kinetic energy of ejected matter is the difference between the thermonuclear energy released and the net binding energy of the white dwarf. Nuclear conversion of C–O to ^{56}Ni produces 1.4×10^{51} erg $M_\odot^{-1}$. Detailed hydrodynamic calculations[6,7] for exploding white dwarfs show, however, that less than half of the material actually becomes ^{56}Ni. Some of the energy release is associated with the production of other elements. Calculations, which treat the nonradial flow in a crude way but the nucleosynthesis in detail[8,9], give a similar result; 3.1×10^{51} erg of nuclear energy is released per solar mass of synthesized ^{56}Ni. The net binding energy is 0.5×10^{51} erg, so $E_\kappa 2/10^{51} = 3.1\ M_{Ni} - 0.5$. Note that E_κ depends strongly on M_{Ni} for $M_{Ni} < 0.4\ M_\odot$, and goes to zero as M_{Ni} approaches 0.16 $M_\odot$. Numerical simulations[3] have shown that at least 0.4 $M_\odot$ of ^{56}Ni (corresponding to 0.74×10^{51} ergs of nuclear energy) must be synthesized to account for velocities at the photosphere, inferred from optical spectroscopy[5], of 10,000–12,000 km s^{-1} during the first few weeks after maximum light. We regard $M_{Ni} > 0.4\ M_\odot$ as a conservative lower limit because, in the context of the model, it imples that, although another $\approx 0.4\ M_\odot$ is burned to other species, 0.6 $M_\odot$ of C–O is not burned at all. Smaller amounts of C–O in the ejecta are indicated by both the optical[5] and X-ray spectroscopy (C. Sarazin, personal communication). Taking all available evidence, we consider the best current estimate for the nickel produced to be $M_{Ni} = 0.6\ M_\odot$ (1.86×10^{51} ergs of nuclear energy). Thus, our best estimate of L_{max} is 1.3×10^{43} erg s^{-1}, with limits of 3.05×10^{43} (1.4 $M_\odot$ Ni) and 0.87×10^{43} erg s^{-1} (0.4 $M_\odot$ Ni).

The transformation from bolometric luminosity L_{max} to absolute blue magnitude M_B, required for comparison with observed SN I, is straightforward[3,10]. At the time of maximum luminosity, the observed flux distribution for $\lambda > 0.4\ \mu$m resembles that of a 20,000 K blackbody, while only a small fraction of the flux is emitted at $\lambda < 0.4\ \mu$m (ref. 5). When the model luminosity is distributed in this way, we find $M_B = 19.23 - 2.5 \log L_{max}/10^{43}$. Note that the colour temperature enters only as a convenient parameter for distributing the model luminosity over wavelength, and that the relation between L_{max} and M_B depends only weakly on its value. An understanding of the atmospheric processes which determine the flux distribution is not required for our present purpose. We simply use the observed distribution. The best estimate of M_B is -19.5, with limits of -20.4 and -19.1.

The predicted values of M_B are related to H_0 through the observed dependence of the apparent magnitudes of SN I on the redshifts of their parent galaxies. To bypass the issues of interstellar extinction in the parent galaxies and departures from ideal Hubble flow in the local Virgo supercluster we restrict our attention to SN I in elliptical and lenticular galaxies having recession velocities >3,000 km s^{-1}. There are six such SN I (including three in the Coma cluster) having well-determined apparent magnitudes at peak. After allowing for interstellar extinction in our Galaxy[11], using $M_B = M_{pg} + 0.3$ (ref. 12) when necessary, and allowing for a difference of 0.1 magnitude between the apparent blue magnitude at 15 (optical peak) and 17 days (bolometric peak) after the explosion we find $M_B = -18.4 \pm 0.2 + 5 \log(H_0/100)$, in good agreement with calibrations done previously[12,13]. The predicted values of M_B then correspond to a best estimate of $H_0 = 59$ km s^{-1} Mpc^{-1}, a rigorous lower limit of 39 km s^{-1} Mpc, and a conservative upper limit of 73 km s^{-} Mpc^{-1}. The uncertainties in the time of bolometric maximum and in the absolute magnitude calibration give an r.m.s. uncertainty for each of these values of $\leq 10\%$. This is small compared with the uncertainty associated with the ejected nickel mass.

The arguments expressed above can be summarized in the relationship $H_0 = 46 \pm 4\ M_{Ni}^{-1/2}$ km s^{-1} Mpc^{-1}. We have argued that values of H_0 in excess of about 73 km s^{-1} Mpc^{-1} would require $M_{Ni} < 0.4\ M_\odot$, and that the corresponding kinetic energy and velocities would be too small. A high value of H_0 could be reconciled with exploding white dwarfs as SN I if only a portion of the matter is ejected, with the remainder forming a neutron star. Kinetic energy and nickel mass would become uncoupled because some kinetic energy would come from the binding energy of the neutron star. Studies of galactic remnants of SN I indicate, however, that there is no evidence that neutron stars are left behind[14,15].

The discussion above has rested on the tacit assumption that all SN I have identical properties. Observationally, this is a tenable proposition, except for a few supernovae (SN 1885a, 1954a, 1962l, 1964l, 1980i, 1983n) which have shown definite spectroscopic and/or photometric peculiarities, and theoretically it is attractive because the ignition of carbon necessarily occurs near the Chandrasekhar mass. There is some evidence, however, for a correlation between peak absolute magnitude and the rate of decline of the initial post-peak light curve[16–18].

If the peak luminosities of normal SN I do cover a finite range, the values of H_0 quoted here are likely to be too high, because, owing to observational selection effects, the SN I beyond the local supercluster will tend to be more luminous than the much nearer SN I, such as 1972e in NGC5253 and 1981b in NGC4536, whose characteristics are used to estimate the nickel mass.

These results are entirely independent of methods for determining distance to Type II supernovae, which involve an understanding of the atmosphere and spectral line-forming regions. In addition, although model dependent, this method is independent of the traditional 'distance ladder' approach.

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1. Wagoner, R. V. *Astrophys. J.* **250**, L65–L69 (1981).
2. Arnett, W. D. *Astrophys. J.* **253**, 785–797 (1982).
3. Sutherland, P. G. and Wheeler, J. C. *Astrophys. J.* **280**, 282–297 (1984).
4. Barbon, R., Ciatti, F. & Rosino, L. *Astr. Astrophys.* **25**, 241–248 (1973).
5. Branch, D. *et al. Astrophys. J.* **270**, 123–139 (1983).
6. Müller, E. & Arnett, W. D. *Astrophys. J. Lett.* **261**, L109–L115 (1982).
7. Müller, E. & Arnett, W. D. in *Nucleosynthesis: Problems and Challenges* (eds Arnett, W. D. & Truran, J. W.) (University of Chicago, in the press).
8. Nomoto, K., Thielmann, F.-K. & Yokoi, K. *Astrophys. J.* **268**, 644–658 (1984).
9. Woosley, S. E., Axelrod, T. S. & Weaver, T. A. in *Stellar Nucleosynthesis* (eds Chiosi, C. & Renzini, A.) (Reidel, Dordrecht, 1984).
10. Schurmann, S. R. *Astrophys. J.* **267**, 779–794 (1983).
11. Burstein, D. & Heiles, C. *Astrophys. J.* **225**, 40–55 (1978).
12. Branch, D. & Bettis, C. *Astr. J.* **83**, 224–227 (1978).
13. Tammann, G. A. in *Supernovae: A Survey of Current Research* (eds Rees, M. J. & Stoneham, R. J.) 371–403 (Reidel, Dordrecht, 1982).
14. Helfand, D. J. in *Type I Supernovae* (ed. Wheeler, J. C.) 20–24 (University of Texas, Austin, 1980).
15. Nomoto, K. & Tsuruta, S. *Astrophys. J. Lett.* **250**, L19–L23 (1981).
16. Rust, B. W. thesis, Univ. Illinois, Urbana (1974).
17. Pskovskii, Y. P. *Soviet Astr.-A. J.* **21**, 675–682 (1977).
18. Branch, D. *Astrophys. J.* **258**, 35 (1982).

Hydrated interplanetary dust particle linked with carbonaceous chondrites?

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Recent mineralogical and geochemical studies of interplanetary dust particles (IDPs) provide convincing evidence that they contain materials that formed in the early solar nebula. Some chondritic IDPs contain hydrated phases and may be related to CI and CM carbonaceous chondrites. However, detailed mineralogical data of the hydrated IDPs are extremely limited. We report here the results of transmission electron microscope (TEM) observations of a hydrated IDP containing Fe-, Mg-rich smectite or mica as a major phase. The sheet silicate appears to have formed by alteration of anhydrous silicates. Fassaite, a Ca, Al clinopyroxene, also occurs in this particle, and one of the crystals exhibits solar-flare tracks, clearly indicating that it is extraterrestrial. Fassaite is a major constituent of the Ca-, Al-rich refractory inclusions (CAIs) found in the carbonaceous chondrites. Its presence in this particle, therefore, suggests that there may be a link between hydrated IDPs and carbonaceous chondrites in the early history of the Solar System.

The hydrated particle studied here, 'Skywalker' (NASA no. r21-M3-5A), was collected from the upper stratosphere by NASA U-2 aircraft. Sandford *et al.*[1] showed that its infrared spectrum contains absorption bands at ~3,400 cm^{-1} and ~1,600 cm^{-1}, which they attributed to the presence of water. A large deuterium enrichment, measured with the ion probe[2], provides strong evidence that Skywalker contains a unique isotope record, and this was probably established in or before the early Solar System[3]. Thus, Skywalker is of great interest, particularly regarding its relations to the carbonaceous chondrites.

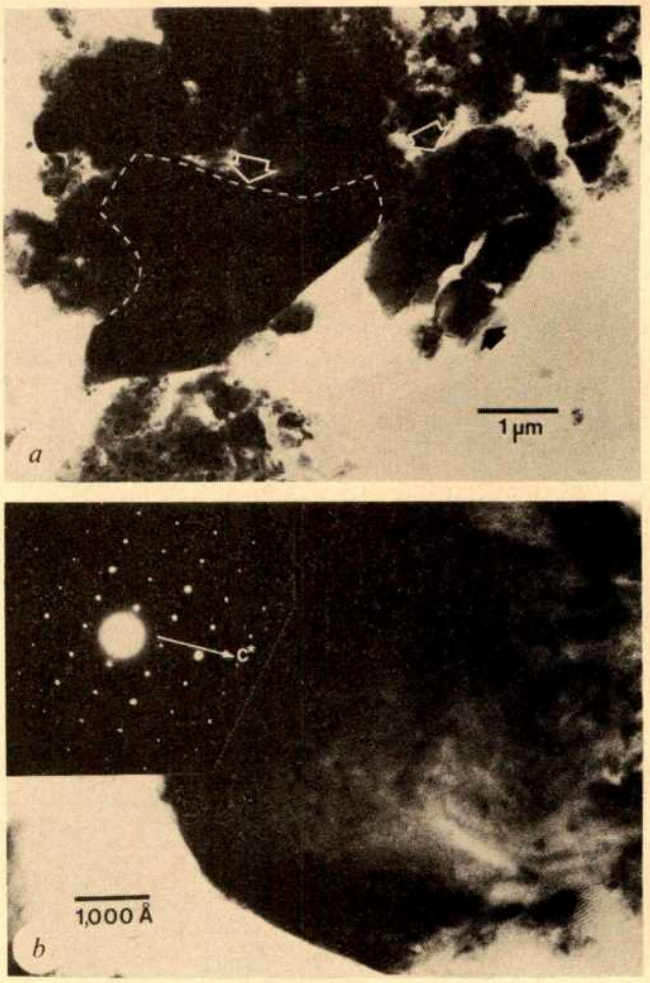


Fig. 1 *a*, Fassaite grains (indicated by arrows) in one of the Skywalker aggregates; surrounding them are clusters of the smectite/mica and pyrrhotite grains. The clustered grains on the right are apparently broken fragments of a single grain, ~2.5 μm in diameter. The grain on the left is the largest single crystal found in Skywalker. *b*, Magnified image of the fassaite grain indicated by a small arrow in *a*, showing solar-flare tracks. The SAED pattern in the inset, obtained from the same grain, corresponds to [101] of clinopyroxene.

TEM imaging and chemical analysis by energy-dispersive X-ray spectroscopy (EDS) have been used to study the microstructures and compositions of the constituent minerals of Skywalker. Structural identifications were made by selected-area electron diffraction (SAED), convergent-beam electron diffraction and high-resolution imaging. The analyses of individual grains were performed using a finely focused beam (~800 Å in diameter). Quantitative data were derived for silicate minerals using thin-film X-ray microanalysis[4]. Details of the experimental procedure and reference standards for the quantitative analyses are given elsewhere[5].

A portion of Skywalker was placed on a carbon film, then mounted on a TEM grid. The sample on the grid consists of six aggregates, ranging from 4 to 7 μm in size, and numerous finer grains. EDS spectra, obtained using a broad beam, show that the particle falls into the chondritic subset of Brownlee[6]. Based on its infrared spectrum, Sandford *et al.*[1] placed Skywalker in the small group of hydrated IDPs.

The pyroxene occurs as a cluster of five grains that range up to 4.5 μm in diameter; four are apparently broken fragments of a single crystal (Fig. 1). Their SAED patterns correspond to clinopyroxene. The grains contain major amounts of Mg, Al

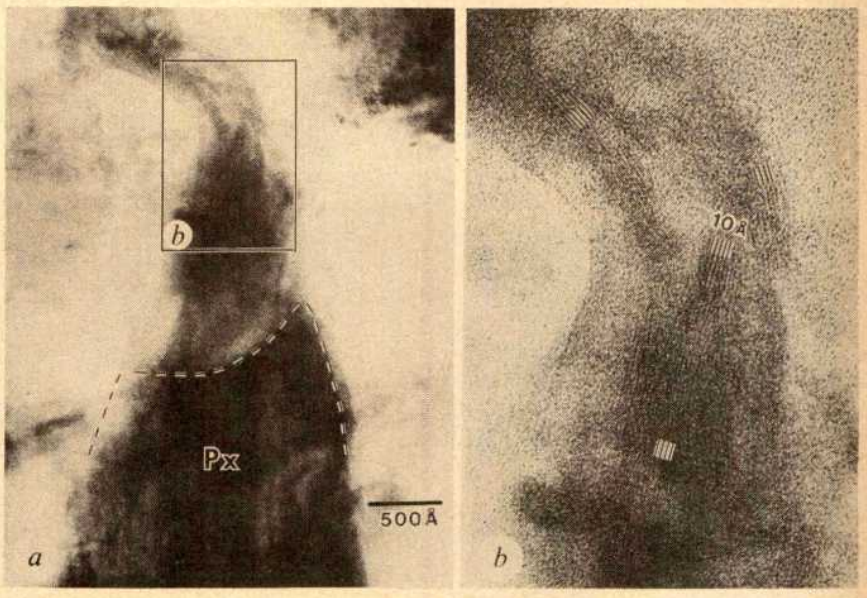


Fig. 2 *a*, A low-magnification TEM image of an enstatite grain. Above the dashed line is the phyllosilicate. *b*, A high-resolution TEM image of the phyllosilicate from the boxed area in *a*, displaying the curved fringes with ~10-Å spacing.

and Ca and minor amounts of Ti, Cr and Fe (Table 1). Using the ratios given in Table 1, the proposed chemical formula is $[Ca_{0.7}Mg_{0.8}Al_{0.4}](Si_{1.8}Al_{0.2})O_6$, which is consistent with its designation as fassaite[7].

Fassaite occurs in both terrestrial and meteoritic samples. It is of special interest in meteorites because it is a major constituent of the primitive and refractory CAIs that are prominent in the CV carbonaceous chondrites[8,9] and that also occur in the CM[10,11] and ordinary chondrites[12,13]. Fassaite in CAIs shows a wide range of Al content (9–22 wt% Al_2O_3) and Ti content (2–18 wt% TiO_2), but it is commonly enriched in both these elements and depleted in Fe relative to fassaite in terrestrial samples[8,9]. The fassaite in Skywalker is aluminous, low in Ti and Fe (Table 1). The high Al and low Fe contents distinguish Skywalker fassaite from terrestrial fassaite[7]. Furthermore, the limited compositional similarity between the fassaite in the IDP and the carbonaceous chondrites raises at least the possibility that there is a genetic relationship between the two occurrences.

Fossil nuclear tracks occur in one of the fassaite grains (Fig. 1*b*). Such tracks in IDPs were first described from olivines and pyroxenes in ordinary chondritic IDPs by Bradley *et al.*[14]. The tracks probably resulted from IDP exposure to solar cosmic rays before capture by the Earth[15]. Laboratory experiments show that nuclear tracks in silicates can be annealed by pulse (2 s) heating to 600 °C (ref. 15). Thus, the tracks provide evidence both that fassaite in Skywalker is extraterrestrial and that it has not been severely heated since track implantation, even during atmospheric entry.

Table 1 Thin-film, X-ray microanalyses (oxide weight percentages and cation ratios per six oxygens) of fassaite from the Skywalker IDP and microprobe analyses of fassaites from meteoritic CAIs and eclogite

	Skywalker*	Allende[9]	Murchison[11]	Eclogite[33] (terrestrial)
Oxide (wt%)				
MgO	14.9 (1.2)	11.51	12.34	15.13
Al_2O_3	14.5 (0.8)	15.14	15.91	7.41
SiO_2	49.6 (1.9)	42.90	42.41	50.85
CaO	18.7 (1.2)	26.02	24.34	17.37
TiO_2	0.6 (0.1)	6.01	3.53	0.78
Cr_2O_3	0.5 (0.1)	—	—	—
FeO	1.2 (0.7)	0.03	—	5.99
Total	100.0	101.58	98.53	97.53†
No. of tetrahedral cations on the basis of six O				
Si	1.75	1.54	1.56	1.84
Al	0.25	0.46	0.44	0.16
Total	2.00	2.00	2.00	2.00
No. of other cations				
Al	0.35	0.18	0.26	0.16
Cr	0.01	—	—	—
Ti	0.02	0.16	0.10	0.02
Mg	0.79	0.62	0.68	0.82
Fe	0.04	0.00	—	0.18
Ca	0.71	1.00	0.96	0.67
Total	1.92	1.96	1.99	1.85

* Average of analyses from 12 different points. Values in parentheses are standard deviations. Original data were obtained in relative ratios, then normalized to 100%.

† Na, K and Mn are excluded.

Both Skywalker and Low-Ca[5], which is the only other hydrated IDP that has been studied by TEM, contain a mineral that has a distinctly layered appearance in TEM images. It contains Si, Fe, Mg, Al and has an interlayer spacing of 10–12 Å; average cation/silicon ratios for the mineral in Skywalker are 0.30 (Mg/Si), 0.28 (Fe/Si) and 0.08 (Al/Si), and for the mineral in Low-Ca they are 0.49 (Mg/Si), 0.26 (Fe/Si) and 0.09 (Al/Si). Based on these criteria, plus the infrared evidence for hydrated phases, we[5] have identified it in Low-Ca as an Fe-, Mg-rich smectite or mica, which we hereafter call smectite/mica. The same identification also holds for Skywalker.

Skywalker also contains small amounts of an Fe-rich phyllosilicate; average cation/silicon ratios are 0.21 (Mg/Si), 0.79 (Fe/Si) and 0.09 (Al/Si). Both smectite/mica and Fe-rich phyllosilicate occur as aggregates of poorly crystalline, fine fibres similar to those illustrated in ref. 5. As in Low-Ca, smectite/mica occurs in close association with Fe–Ni sulphides, mostly rounded grains (~1,000 Å) of nickeliferous pyrrhotite, whereas the Fe-rich phyllosilicate is rarely associated with sulphides. The Fe-rich phyllosilicate exhibits weak powder electron-diffraction lines at ~4.6, ~2.6 and ~1.5 Å. Its poor crystallinity hindered further structural characterization. Smectite/mica in Skywalker is commonly more Fe-rich and Mg-poor and shows a wider variation in these elements than that in Low-Ca. This variation probably results from partial mixing with the Fe-rich phyllosilicate.

Platelets of enstatite pyroxene and forsterite olivine, containing up to a few atomic per cent of Fe and ranging in diameter from 0.1 to 3 μm, are common in Skywalker, in marked contrast to Low-Ca, where olivine is rare and no pyroxene was found[5]. Olivine platelets commonly display strong strain-field contrast and complex patterns of Moiré fringes. Pyroxene platelets typically show a disordered stacking of clino- and ortho-enstatite[16,17] (Fig. 3). Similar platelets of olivine and pyroxene are also common in anhydrous IDPs[18,19]. Bradley *et al.*[19] suggested that such pyroxene platelets are probably primary vapour-phase condensates that could have formed in the primitive solar nebula.

Figure 2 shows a grain consisting of Mg–Fe pyroxene at one end and phyllosilicate at the other. The high-resolution TEM image (Fig. 2*b*) displays curved fringes characteristic of phyllosilicate and having a spacing of ~10 Å. EDS analysis indicates

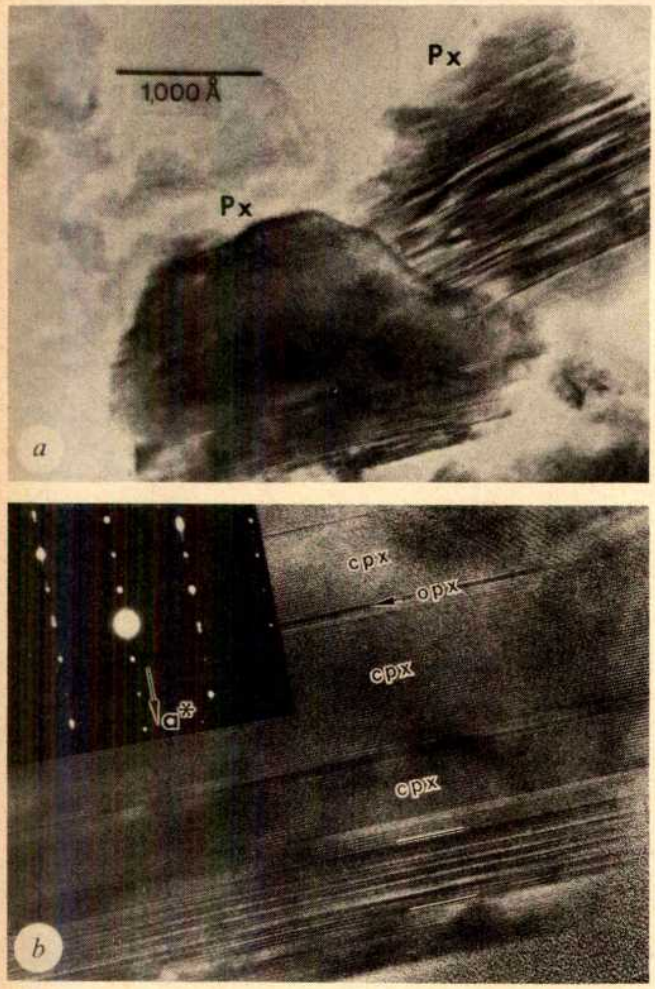


Fig. 3 *a*, Enstatite platelets. *b*, A high-resolution TEM image of part of the pyroxene grain at the borrom of *a* and its SAED pattern (inset). The image shows regions of clinopyroxene (cpx), orthopyroxene (opx) and stacking disorder of cpx and opx (between lines); cpx and opx have fringe spacings of 9 and 18 Å. Streaking of reflections along a* in the diffraction pattern results from stacking disorder of cpx and opx.

that the phyllosilicate is much richer in Fe than the pyroxene; Al was not detected from either the pyroxene or the phyllosilicate. Although the actual boundary between the two minerals is poorly defined, the external edge is clearly continuous around both minerals, and the pyroxene and the phyllosilicate are certainly parts of the same grain. Many small grains of smectite/mica also occur along the edges of other pyroxene crystals. By analogy with images obtained of terrestrial[20,21], meteoritic[22] and synthetic samples[23], we believe that the phyllosilicate was produced by alteration of the pyroxene. It is, therefore, reasonable to assume the past occurrence of aqueous alteration in the history of this IDP, although it is not possible to say whether liquid or vapour was responsible for the alteration.

An unidentified mineral containing major Fe, Mg and minor Ca, Mn and Si is widespread and is commonly associated with smectite/mica; it occurs in rounded grains, 500 Å to 1 μm in diameter. A similar phase was also found in Low-Ca[5], but it has not been reported from either other IDPs or meteorites and so may be unique to the hydrated IDPs.

Fassaite has not been reported previously from IDPs; its discovery provides important information regarding the origin of the hydrated IDPs. The bulk compositions of the chondritic IDPs as well as their isotope compositions and mineralogy suggest a strong similarity between IDPs and carbonaceous chondrites. If fassaite in Skywalker formed by processes related to the formation of CAIs, then its presence provides evidence suggesting that the hydrated IDPs contain primitive materials that formed in the early solar nebula. The hydrated IDPs, like carbonaceous chondrites, presumably have not experienced extensive high-temperature metamorphism since their accretion.

Although there are definite mineralogical similarities between hydrated IDPs and carbonaceous chondrites, our TEM studies also reveal important differences. The phyllosilicates in the CI and CM meteorites are mostly serpentine- or septechlorite-type minerals[24–29] rather than the Fe- and Mg-rich smectite or mica in both Low-Ca and Skywalker. The phyllosilicates in the carbonaceous chondrites are commonly much better crystallized than those in the IDPs. Low-Ca and Skywalker are thus probably not broken fragments of carbonaceous chondrites, and they may well have originated from distinct sources.

Petrographical studies[26–30] suggest that the CI and CM phyllosilicates were produced largely by aqueous alteration of olivines and pyroxenes. The present study provides evidence suggesting that the IDP phyllosilicates were also formed by alteration of Fe-Mg pyroxene. The differences in phyllosilicate mineralogy may then reflect the distinct environments in which the alteration occurred. If the alteration occurred within the parent bodies—and this is only an assumption at present—the contrast in mineralogy may reflect differences between comets and asteroids, the presumptive parent bodies of IDPs and carbonaceous chondrites[31,32].

The hydrated and non-hydrated IDPs have similar bulk compositions and isotope characteristics but different mineralogies. A question arises as to whether the distinct mineralogies reflect different environments. During or after accretion from the solar nebula, the IDPs may have experienced distinct physicochemical conditions or different degrees of thermal metamorphism. To resolve this question, additional mineralogical and isotope data are required as well as a better understanding of the range of types of chondritic IDPs. Work is in progress towards achieving these goals.

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1. Sandford, S. A., Fraundorf, P., Patel, R. & Walker, R. M. *Meteoritics* **17**, 276–277 (1982).
2. Zinner, E. & McKeegan, K. D. *Lunar planet. Sci.* **15**, 961–962 (1984).
3. Zinner, E., McKeegan, K. D. & Walker, R. M. *Nature* **305**, 119–121 (1983).
4. Cliff, G. & Lorimer, G. W. *J. Microsc.* **103**, 203–207 (1975).
5. Tomeoka, K. & Buseck, P. R. *Earth planet. Sci. Lett.* **69**, 243–254 (1984).
6. Brownlee, D. E. in *Protostars and Planets* (ed. Gehrels, T.) 134–150 (University of Arizona Press, 1978).
7. Deer, W. A., Howie, R. A. & Zussman, J. in *Rock Forming Minerals* Vol. 2A, 399–414 (Wiley, New York, 1978).
8. Clarke, R. S. *et al. Smithson. Contr. Earth Sci.* **5**, 1–53 (1970).
9. Grossman, L. *Geochim. cosmochim. Acta* **39**, 433–454 (1975).
10. Macdougall, J. D. *Earth planet. Sci. Lett.* **42**, 1–6 (1979).
11. MacPherson, G. J., Bar-Matthews, M., Tanaka, T., Olsen, E. & Grossman, L. *Geochim. cosmochim. Acta* **47**, 823–839 (1983).
12. Bischoff, A. & Keil, K. *Nature* **303**, 588–592 (1983).
13. Bischoff, A. & Keil, K. *Geochim. cosmochim. Acta* **48**, 693–709 (1984).
14. Bradley, J. P., Brownlee, D. E. & Fraundorf, P. *Science* **226**, 1432–1434 (1984).
15. Fraundorf, P., Flynn, G. J., Shirck, J. & Walker, R. M. *Proc. 11th Lunar. planet. Sci. Conf.*, 1235–1249 (1980).
16. Iijima, S. & Buseck, P. R. *Am. Miner.* **60**, 758–770 (1975).
17. Buseck, P. R. & Iijima, S. *Am. Miner.* **60**, 771–784 (1975).
18. Christoffersen, R. & Buseck, P. R. *Lunar planet. Sci.* **15**, 152–153 (1984).
19. Bradley, J. P., Brownlee, D. E. & Veblen, D. R. *Nature* **301**, 473–477 (1983).
20. Veblen, D. R. & Buseck, P. R. *Am. Miner.* **66**, 1107–1134 (1981).
21. Eggleton, R. A. & Boland, J. N. *Clay Clay Miner.* **30**, 11–20 (1982).
22. Tomeoka, K. & Buseck, P. R. *Nature* **299**, 327–329 (1982).
23. Yada, K. & Iishi, K. *Am. Miner.* **62**, 958–965 (1977).
24. Fuchs, L. H., Olsen, E. & Jensen, K. J. *Smithson. Contr. Earth Sci.* **10**, 1–39 (1973).
25. Tomeoka, K. & Buseck, P. R. *Nature* **306**, 354–356 (1983).
26. DuFresne, E. R. & Anders, E. *Geochim. cosmochim. Acta* **26**, 1085–1114 (1962).
27. Nagy, B., Meinschein, W. G. & Hennessy, D. J. *Ann. N.Y. Acad. Sci.* **108**, 534–552 (1963).
28. Boström, K. & Fredriksson, K. *Smithson. Misc. Collect.* **151**, 1–39 (1966).
29. Bunch, T. E. & Chang, S. *Geochim. cosmochim. Acta* **44**, 1543–1577 (1980).
30. McSween, H. Y. *Geochim. cosmochim. Acta* **43**, 1761–1770 (1979).
31. Brownlee, D. E. *Rev. Geophys. Space Phys.* **17**, 1735–1743 (1979).
32. Gaffey, M. J. & McCord, T. B. in *Comets, Asteroids, Meteorites* (ed. Delsemme, A. H.) 199–218 (University of Toledo Press, 1977).
33. Yoder, H. S. & Tilley, C. E. *J. Petrol.* **3**, 342–532 (1962).

Magnetic reversals and mass extinctions

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Previous analyses of the time distribution of reversals of the Earth's magnetic field have yielded mixed results. Some authors have claimed significant periodicities of order 10^7 yr[1,2] whereas others have reported failure to reject null hypotheses of random spacing at that scale[3–6]. Because of repeated suggestions that field reversal is linked to biological extinction[7–12], further analysis of the magnetic time series is appropriate. I present here the results of a study of the reversal record of the past 165 Myr. A stationary periodicity of 30 Myr emerges (superimposed on the non-stationarities already established by others[5]), which predicts pulses of increased reversal activity centred at 10, 40, 70, ... Myr BP.

The database is the compilation of magnetic reversals for the late Jurassic to present interval (0–165 Myr BP) by Harland *et al.*[13]. These data, constituting the magnetostratigraphical timescale for the best-known part of the reversal record, were culled heavily by Harland *et al.*[13] to eliminate minor excursions and other short-term or local effects and were presented as the time boundaries of 148 magnetic intervals (chrons and subchrons), yielding $148 \times 2 = 296$ reversal events. Figure 1 shows the distribution of these events in geological time, which shows, among other things, the well-known quiet period of the late Cretaceous (83–118 Myr BP), with reversal activity decreasing before and increasing after the quiet period.

In developing the statistical methodology for this analysis, a magnetic reversal is assumed to be an isolated event; thus, it is not important whether the field shifted from normal to reversed or vice versa. The analytical procedure, which follows that of Stothers[14] and Raup and Sepkoski[15], has as a central feature a 'goodness-of-fit' metric applicable to any postulated stationary periodicity. This metric is calculated as follows. For a given trial period (such as 20 Myr), a predicted sequence of events is defined and placed on the timescale in a completely arbitrary phase position (such as −10, 10, 30, 50, ... Myr BP). Then, the fit is assessed as the standard deviation of departures of the observed events from the predicted event closest to each. Thus, in the hypothetical example used here, an event observed at 14 Myr BP would show a departure of +4 Myr from its closest predicted event (10 Myr BP), an observed event at 48 Myr BP would show a departure of −2 (from the 50-Myr BP prediction) and so on. The standard deviation of these departures is a measure-of-fit for a given phase position, which is then shifted by a small increment (to, for example, −9, 11, 31, 51, ... Myr BP) and the standard deviation of departures is recalculated.

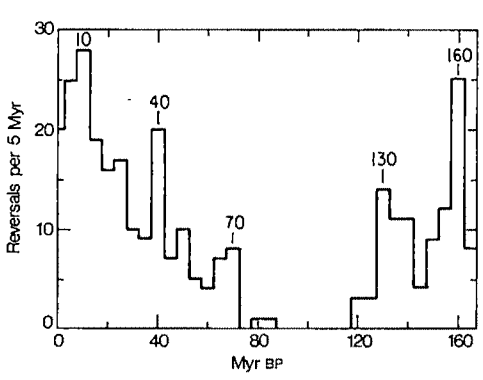


Fig. 1 Frequency of 296 magnetic reversals in 5-Myr intervals (data from ref 13).

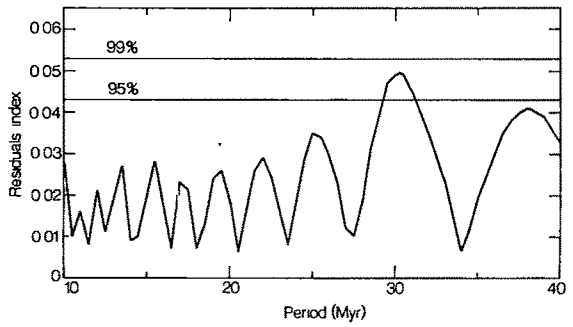


Fig. 2 Spectrum of residuals index ($n = 296$) constructed using the method of Stothers[14] The residuals index is the measure of goodness of fit of the data to a given period.

This process is repeated for a series of phase increments (totaling at least a full period) and the lowest value of the standard deviation is taken as the goodness-of-fit. We thus have estimates of goodness-of-fit and best-fit position for the trial period. The method is particularly appropriate for time series exhibiting missing or missed data because waiting times between events are never examined explicitly. Also, the method contains no assumption that the frequency behaviour is sinusoidal, contrary to conventional Fourier techniques.

Stothers[14] showed that the standard deviation can be normalized for period length as follows. Residuals index

$$(P\sqrt{(n^2-1)/12n^2} - \sigma)/P$$

where P is the period length, n is the number of observations and σ is the standard deviation of departures (above). With random data, the residuals index is constant over a range of P (but see below).

Given an observed time series, the residuals index may be calculated for a sequence of P values and a spectrum of goodness of fit produced, which is shown in Fig. 2 for the magnetic reversal time series; periods greater than 40 Myr are not included because the residuals index is unstable above $P = 40$ with this particular time series. There are a number of well-defined peaks, but this is not surprising; indeed, random input of the same general character often gives comparable spectra and so it is important to assess the probability that the spectrum is not just the product of chance. Using a bootstrap technique, the real-time series was randomized by scrambling the intervals between successive magnetic reversals; the first and last reversals were used as fixed endpoints and all intervals in between were rearranged by standard Monte Carlo techniques. Each randomized version of the data thus retained most of the fabric of the true series, but the placement of reversals was changed; each had a quiet period

Table 1 Goodness-of-fit and phase for magnetic reversal data

Period (Myr)	Goodness-of-fit: % of simulations with poorer fit than the real data			Phase in best-fit position, age of cycle no. 1			
	0–165	0–83	118–165	(Myr BP)	0–165	0–83	118–165
22	83.6	43.2	96.8		4 49	4 59	4.07
23	45.8	12.2	100.0		0.05	8.59	20.12
24	64.6	20.2	100.0		12.03	8.38	14.13
25	92.2	31.7	100.0		8.35	8.23	8.60
26	84.2	57.5	100.0		7.12	8.91	3 07
27	29.5	75.0	99 9		5.15	7.93	24.27
28	65.1	81.1	99 9		15.03	7.03	19.45
29	98.0	81.7	99.7		12.16	10.62	14.90
30	99.4	82.9	99.6		10.04	10.04	10 05
31	98 9	80.4	99.0		7.80	9.77	5 18
32	96.8	77.5	97.6		5.11	9.20	0 93
33	83.0	74.8	96.5		3.93	8.97	29 04

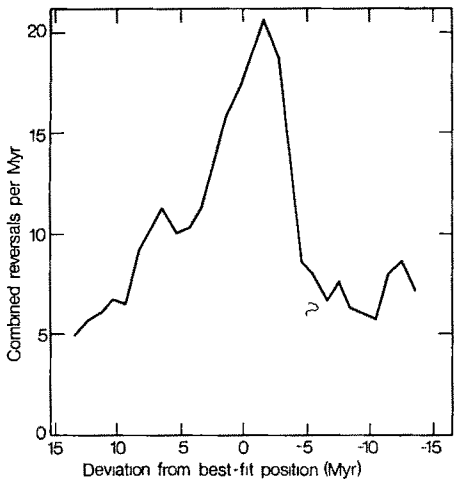


Fig. 3 Composite cycle of magnetic reversal activity plotted as a 3-point moving average. With a 30-Myr period in best-fit position (zero on the horizontal axis), the data from all cycles have been superimposed ($n = 296$).

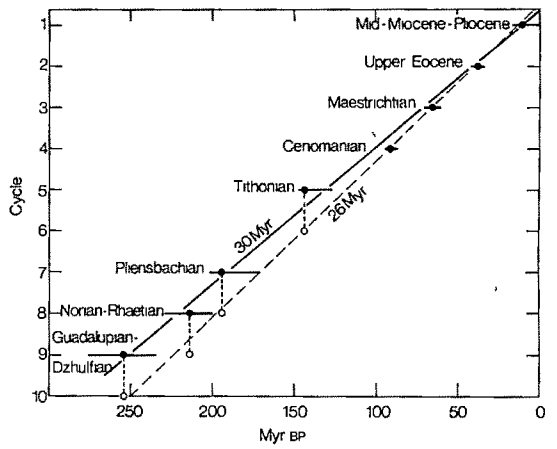


Fig. 4 The distribution by age and cycle number of the eight significant biological extinctions of the last 250 Myr (solid dots). Error bars indicate the maximum possible age spread, including that implied by the several available geological timescales[25]. The solid diagonal corresponds to a 30-Myr period in the best fit position indicated by the magnetic reversal data. If the 26-Myr period is used, the oldest four extinctions must be advanced one cycle each (open circles) and the fit is improved substantially (dashed diagonal).

of the same length, but its position was unpredictable. A spectrum, such as that in Fig. 2, was calculated for each of 200 simulations and the point of highest residuals index for each simulation was recorded. The results are summarized by the two horizontal lines in Fig. 2; 95 and 99%, respectively, of the simulated spectra were contained totally below the designated lines.

The peak corresponding to a period of 30 Myr stands out and is clearly above the 95% line, from which it can be concluded that it is unlikely that the peak is an accident or artefact of the method (Fig. 2). The null hypothesis in this case comes from the above randomization scheme. It is non-Poisson in that the distribution of intervals (waiting times) between events is maintained; only their order has been randomized. Tests with two alternative null hypotheses will be discussed below.

With the 30-Myr peak in Fig. 2 as a starting point, a more detailed investigation of periods in that region was undertaken. For each 1-Myr interval from 22 to 33 Myr, the fit of the real data (measured by the standard deviation of departures) was compared with that of 1,000 simulations of the type used earlier. The results are shown in columns 2 and 5 of Table 1. In column 2, goodness-of-fit is expressed as the percentage of simulations showing a poorer fit (higher standard deviation); the period at 30 Myr is the highest with 99.4%. Column 5 gives the best-fit position expressed as the geological age (in Myr BP) of the most recent pulse of reversal activity and this position for the 30-Myr period is 10.04 Myr BP.

To check these results, the analysis was repeated with non-overlapping segments of the time series, using the Cretaceous quiet period as the demarcation. These results are shown in the remaining columns of Table 1. For the younger segment (0–83 Myr BP), goodness-of-fit values are not as high as for the whole series but the one for 30 Myr is the highest. Also, the best-fit position is identical at 10.04 Myr BP. For the older segment (118–165 Myr BP) all periods show excellent fit, making it difficult to distinguish between them. This is not unreasonable because this segment is only 45 Myr long and contains only two of the events predicted by the complete time series. Note, however, that the best-fit position for the 30-Myr period estimated from this short segment is 10.05 Myr BP, essentially identical to the other estimates for this period. Thus, the analyses of segments can be regarded as confirming a 30-Myr period with a best-fit phase position of 10 Myr BP.

I conclude that the reversal time series probably shows a stationary periodicity predicting elevated reversal activity at 30-Myr intervals beginning at 10 Myr BP. Extrapolating from this conclusion, Fig. 1 shows that the record is dominated by the Cretaceous quiet period with reversal activity increasing in both directions. The 30-Myr periodicity is superimposed on this broader first-order change and can be seen as localized peaks at 10, 40, 70, 130 and 160 Myr BP; the predicted peak at 100 Myr BP is missing because of overall reduction in reversal activity (the quiet period).

I emphasize that a statistical confidence level cannot be assigned to the 30-Myr periodicity. Although the data clearly favour this periodicity over the null hypothesis of random ordering of the same data, an unknown number of stationary and non-stationary models might give as good or a better fit. The 30-Myr stationary model is, nevertheless, the simplest alternative to the random model and seems to be well supported by the data.

Confidence in the 30-Myr periodicity depends on the null hypothesis against which it is tested; it was therefore tested against two other hypotheses. The first is a simple Poisson model where randomization was achieved by 'dropping' 296 points on a 0–165-Myr timescale using uniformly distributed random numbers; the result was identical, within sampling error, to that reported in Table 1. The second variant reproduced the non-stationarities reported by McFadden and Merrill[5]. Once again randomization was achieved by a Poisson method, but in this case the linear decrease in reversal activity before the Cretaceous quiet period and the linear increase after that were used to bias the selection of simulated events. The probabilities used were drawn from linear fits to the actual data following the interpretation of McFadden and Merrill[5]. The result, identical to that reported in Table 1 (99.4% of simulations showing a poorer fit than the real data), confirms further the conclusion that the observed periodicity is superimposed on the larger-scale changes in reversal frequency.

Figure 3 shows the frequency distribution of reversal events in a single composite 30-Myr period. The relatively smooth and symmetrical distribution supports the hypothesis of a 30-Myr period with a best-fit position centred at 10, 40, 70, ... Myr BP. If other periods or phase positions are used to construct the composite, the distribution breaks down.

These results are in good agreement with other analyses[1,2]. Using Walsh spectrum analysis applied to a much longer series of magnetic reversal data, Negi and Tiwari[1] recognized several periodicities and claimed statistical significance for those at 32 and 285 Myr. Mazaud *et al.*[2], working with the reversal data younger than the Cretaceous quiet period, found a 15-Myr periodicity, which is precisely in phase with the 30-Myr period reported here. Their strongest peaks occurred at 10 and 40 Myr BP and it is not clear (nor especially important) whether the 30-Myr period is a harmonic of the 15-Myr period or vice versa.

What is the relevance of these results to biological extinction? This question involves possible relationships between extinction, comet or asteroid impact and magnetic reversal as well as possible periodicities in all three. The link between extinction and magnetic reversal is supported by some studies[7-12] but challenged by others[16-17]; that between extinction and impact is more nearly established because of the iridium and/or other evidence for impact at four separate extinction times—late Eocene[18-19], terminal Cretaceous[20-21], terminal Permian[22] and late Devonian[23]. Further, the suggestion that extinctions and impact events are both periodic and in phase[12,24-26] strengthens the case for causal linkage. A proposed link between large-body impact and magnetic reversal is more tenuous, but is supported by some evidence, specifically the temporal coincidence of magnetic reversals with three of the four well-dated tectite-strewn fields[27]. It is thus not impossible that at least some reversals are caused by comet or asteroid impact and it is in this context that the relationship between periodic intensification of reversal activity and periodic extinction becomes important.

For the most recent several cycles, the fit between extinctions and reversal intensity is reasonable in view of the extinctions at 11.3, 38 and 65 Myr BP[25] and the magnetic reversal peaks at 10, 40 and 70 Myr BP. But because the most probable periodicity for extinctions is 26 Myr[15,25], the two systems move out of phase going backwards in time. This is illustrated in Fig. 4, which shows an attempt to fit the 30-Myr periodicity to the eight significant extinctions of the past 250 Myr and shows also the 26-Myr fit to the same extinction points. Except for the first three events, the discrepancy between the two periods is clearly non-trivial.

The discrepancy can mean either there are independent periodicities operating with no causal connection or that the statistical uncertainty of the analyses is large enough to accommodate a single period somewhere in the 26–30-Myr range. The situation is complicated further by the fact that Rampino and Stothers[12] re-analysed the extinction data and found a best-fit period of 30 ± 1 Myr, with phase position at 10 ± 7-Myr BP. They used a regression method that assumes that all cycles are present in the data, although they noted that removing this restriction restores the 26-Myr period of Raup and Sepkoski[15,25].

The discrepancies in analytical results mean that no unequivocal conclusion can be drawn; however, there is a possibility that biological extinction, magnetic reversal and large-body impact are linked.

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1 Negi, J G & Tiwari, R K *Geophys Res Lett* **10**, 713-716 (1983)
2 Mazaud, A, Laj, C, de Sèze, L & Verosub, K L *Nature* **304**, 328-330 (1984)
3 Phillips, J D & Cox, A *Geophys J R. astr Soc.* **45**, 19-33 (1976)
4 Cox, A *Phys Earth Planet Interiors* **24**, 178-190 (1981).
5 McFadden, P L & Merrill, R T *J geophys Res* **89**, 3354-3362 (1984)
6 McFadden, P L *Nature* **311**, 396 (1984)
7 Uffen, R J *Nature* **198**, 143-144 (1963)
8 Simpson, J F. *Bull geol Soc. Am.* **77**, 197-204 (1966)
9 Hatfield, C B & Camp, C J *Bull geol Soc. Am* **81**, 911-914 (1970)
10 Hays, J D *Bull geol Soc. Am* **82**, 2433-2447 (1971)
11 Crain, I K *Bull geol Soc. Am* **82**, 2603-2606 (1971)
12 Rampino, M R & Stothers, R B *Nature* **308**, 709-712 (1984)
13 Harland, W B *et al A Geologic Time Scale* (Cambridge University Press, 1982)
14 Stothers, R B *Astr Astrophys* **77**, 121-127 (1979)
15 Raup, D M. & Sepkoski, J J *Proc. natn Acad Sci. USA* **81**, 801-805 (1984)
16 Thomson, K S in *Patterns of Evolution* (ed Hallam, A) 377-404 (Elsevier, Amsterdam, 1977)
17 Plotnick, R E *Geology* **8**, 578-581 (1980)
18 Ganapathy, R *Science* **216**, 885-886 (1982)
19 Alvarez, W, Asaro, F, Michel, H V. & Alvarez, L W *Science* **216**, 886-888 (1982)
20 Alvarez, L W, Alvarez, W, Asaro, F & Michel, H V *Science* **208**, 1095-1108 (1980)
21 Alvarez, W *et al Science* **223**, 1135-1141 (1984)
22 Sun, Y *et al* in *Developments in Geoscience*, 235-245 (Academia Sinica, Science Press, Beijing, 1984)
23 Playford, P E, McLaren, D J, Orth, C J, Gilmore, J S & Goodfellow, W D *Science* **226**, 437-439 (1984)
24 Alvarez, W & Muller, R A *Nature* **308**, 718-720 (1984).
25 Sepkoski, J J & Raup, D M in *Dynamics of Extinction* (ed Elliott, D) (Wiley, New York, in the press)
26 Rampino, M R & Stothers, R B *Science* **226**, 1427-1431 (1984)
27 Glass, B P, Swincki, M B & Zwart, P A *Proc. 10th Lunar Planet Sci. Conf* 2535-2545 (1979)

Lead isotope differences between whole-rock and phenocrysts in recent lavas from southern Italy

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In southern Italy, there are several active volcanoes which occur in diverse geotectonic settings. Isotope determinations on coexisting minerals and whole-rocks from zero-age lavas from Vesuvius, Etna and Stromboli reveal an isotope equilibrium for Sr isotopes[1] but not for Th (ref. 2). Analyses for Pb in the same samples also reveal differences in isotope composition, and in all cases the phenocrysts are less radiogenic than the whole-rocks. We argue here that the Pb and Th isotope composition of the magmas changed during and after fractional crystallization, possibly by crustal assimilation or by addition of mantle-derived fluids and that whole-rock Pb isotope data are not representative of the magma sources.

Stromboli forms part of the Aeolian island arc, probably along a destructive margin between the European and African plates. It is characterized by virtually uninterrupted eruption of leucite tephrites and basaltic andesites belonging to the high-K-calc-alkaline series. Model calculations based on trace elements indicate an origin by partial melting of a parent material inhomogenously enriched by a Th/light rare-earth element/P-rich metasomatic fluid, probably derived from the subducting plate[3].

Etna is a large alkali basalt volcano, with minor early tholeiitic lavas, producing mainly hawaiites and mugearites. Its geochemistry is largely consistent with its within-plate setting but a minor modification of its source by subduction-derived phases is likely[3].

Vesuvius is best known for its explosive-effusive eruptions of high-K trachytes and leucite tephrites; they probably derive from a comparatively recently enriched mantle source, by analogy with nearby Roccamonfina[4]. Fractional crystallization and cumulus formation took place in various magma chambers at different levels in the crust; the incorporation of limestone fragments, high in the crust, had little effect on the volcanic rocks.

Table 1 shows the results of our analyses of the Pb isotope composition of whole-rock and mineral separates of lavas and ejected cumulates of well-studied samples. Separated minerals are either phenocrysts, considered to have crystallized from the coexisting magma, or biotite separated from nodules which have been interpreted as cumulates[5].

Pb was separated using a 0.1-ml AG 1×8 ion-exchange column, eluted with 1M HBr and stripped with 2 ml H_2O. Pb was loaded on a Re filament with phosphoric acid and silica gel. Isotope ratios were measured using our software on a VG54E mass spectrometer. The NBS 981 standard was run in the same conditions and from the collated results a bias factor of 1.00047 AMU^{-1} was calculated. Measured results were corrected using this factor to minimize the effects of instrumental mass fractionation. The total procedure blank is <2 ng; sample weights were adjusted to contain an estimated 1 μg Pb.

Our data for the Vesuvius 1944 lava are in agreement with Vollmer and Hawkesworth[6], while those from the Etna lavas compare well with the data of Carter and Civetta[7]. Reproducibility may be estimated from the two duplicate whole-rock analyses.

There are no significant differences in Sr isotope ratios (Table 1) between phenocrysts and their whole-rocks, indicating that phenocrysts are indeed co-magmatic. Differences in Sr isotopes between Vesuvius samples from different magma batches can be explained by mixing two endmembers with very similar major-element, but different isotope, compositions[1].

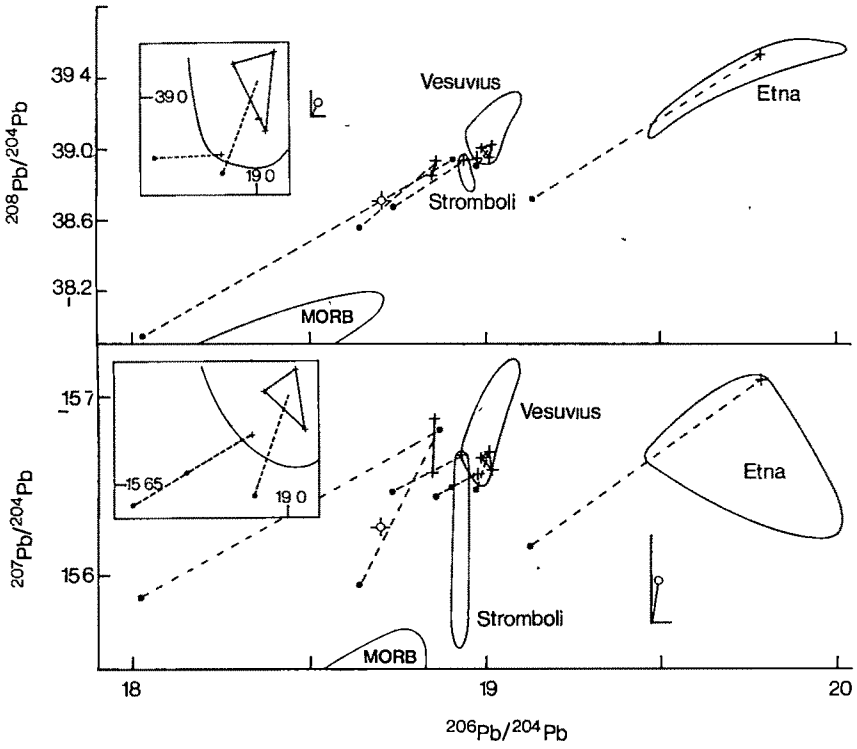


Fig. 1 Pb isotope diagram. Solid lines connect whole-rock duplicates; dashed lines tie whole-rock and mineral. Inset, Vesuvius lava data on a larger scale. ⊹, Average modern Pb[16]. ⌊, The error on replicate analyses of NBS 981 and the applied correction. Top of MORB field from ref. 17.

Pb isotope compositions are plotted in Fig. 1 together with fields constructed using the present data and some from other workers[6-9]. All phenocryst and cumulate nodule data plot away from the whole-rock fields towards less radiogenic compositions. The largest displacement of plagioclase with respect to the whole-rock from which it was separated is from Etna, and is well outside the experimental error. Deviations for the Stromboli and Vesuvius–Castello di Cisterna pairs are smaller but still significant. Data for the Vesuvius 1944 lava are strictly within error, but display a similar relation.

In the case of the cumulate nodules, there are also large differences between biotite and whole-rock analyses. These data suggest that the Pb isotope composition of the respective magmas changed during and after fractional crystallization, although the Sr isotope composition was not affected. (For Sr, a difference in isotope composition caused by mass-dependent fractionation would be eliminated by the standard correction procedure as its effect is indistinguishable from the instrumental fractionation.) Mass-dependent Pb isotope fractionation effects will be parallel to the NBS 981 correction vector. Clearly, none of the mineral, whole-rock tie-lines are parallel to this vector and the most probable explanation is mixing of two components with different Pb isotope composition. The actual nature of the components is difficult to assess but it can be inferred that the magma source had Pb isotope ratios even less radiogenic than indicated by the phenocrysts or cumulate minerals but probably still more radiogenic than mid-ocean ridge basalts (MORB). Slopes of tie-lines on Fig. 1 between minerals and whole-rocks are ~0.12, suggesting an age of ~2,000 Myr for the radiogenic component. Our data do not indicate whether the mixing process involved bulk assimilation, exchange of Pb through interaction with fluids or addition of Pb by metasomatic fluids in the mantle. However, for Vesuvius, fractional crystallization has probably taken place in the mantle at pressures greater than the stability limit of

Table 1 Pb isotope composition compared with Sr and Th data for various Italian lavas

		$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{206}Pb$	$^{87}Sr/^{86}Sr$	$^{230}Th/^{232}Th$*
Stromboli	WR	18.931±5	15.668±5	38.945±11	0.82768±4	0.70624±4†	0.92±1
1975 lava Stromboli[13]	Plag	18.734±2	15.641±1	38.685±4	0.83490±2	0.70621±4‡	0.92±6
Etna	WR	19.777±3	15.712±3	39.546±9	0.79445±2	0.70350±6	0.98±2
1971 lava Etna[15]	Plag	19.129±20	15.617±14	38.726±40	0.81638±8	0.70343±4	1.34±10
Vesuvius/Somma	WR	19.016±1	15.660±1	39.041±4	0.82353±1	0.70718±3§	0.86±2
	WR	18.978±2	15.667±2	39.030±6	0 82553±2		
1944 lava Vesuvius	WR	19.008±13	15.671±11	38.969±36	0.82431±7		
	Leuc	18.969±7	15.648±6	38.930±18	0 82489±3	0.70718±3§	1.10±9
Castello di Cisterna lava	WR	18.967±1	15.659±1	38.947±4	0.82557±5	0.70761±4§	
	Leuc	18.857±11	15.646±10		0.82971±1	0.70757±6§	
	Leuc	18.906±3	15.652±2	38.944±6	0.82785±2		
Cumulate	WR	18.841±1	15.650±1	38.870±2	0.83065±1	0.70741±5§	1.14±4
N1	WR	18.854±5	19.689±5	38.950±12	0.83215±4		
	Biot	18.636±1	15.595±10	38.571±22	0.83681±4	0.70761±5§	1.25±6
Cumulate	WR					0.70728±5§	1.02±6
N83	Biot	18.028±6	15.588±5	37.947±13	0.86554±3	0.70766±3§	1.33±10
	Biot	18.861±3	15.682±3	38.952±6	0.83148±3		
NBS 981		16.923±6	15.470±7	36.650±16	0.91417±10		
$N=14$							

Errors are 1σ for within-run precision; for NBS 981 it is the standard deviation. Samples are corrected for fractionation using a factor of 1.00047 AMU^{-1} derived from repeated analyses of NBS 981. WR, whole-rock; Plag, plagioclase; Leuc, leucite; Biot, biotite.

* Activity ratios from refs 2, 10, 11. Sr isotope data from † ref. 10, ‡ ref. 14, § ref. 1.

plagioclase[5]. Thus, a mantle component is probably involved. Furthermore the $^{230}Th/^{232}Th$ activity ratios[2,10,11] summarized in Table 1 also show differences in isotope composition between whole-rock and minerals in most cases. Capaldi *et al.*[2] have argued that these differences do not have any significance for the age but indicate open system behaviour, and they propose contamination of the magmas with mantle fluids. This must have been a recent event because the high decay rate of the ^{238}U daughter products[12] involved will establish a new equilibrium in 0.2 Myr.

The observed differences in mineral/whole-rock Pb isotopes also imply that whole-rock data alone are not representative of the magma sources, at least in the cases of Vesuvius, Stromboli and Etna. The observed variations suggest that the magma sources are less radiogenic than the whole-rock and probably even the mineral data.

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1 Cortini, M & Hermes, O D *Contr. Miner. Petrol.* **77**, 47–55 (1981)
2. Capaldi G., Cortini, M & Pece, R. *J volcan geotherm. Res* **14**, 247–260 (1982)
3 Beccaluva, L, Rossi, P L & Serri, G *Earth Evol Sci* **3**, 222–238 (1982)
4 Hawkesworth, C J & Vollmer, R. *Contr Miner Petrol.* **69**, 151–165 (1979)
5 Hermes, O D & Cornell, W C *NSF Final Tech Rep* (Univ Rhode Island, 1978)
6 Vollmer, R & Hawkesworth, C J *Earth planet Sci. Lett* **47**, 91–101 (1980)
7 Carter, S R. & Civetta, L *Earth planet Sci. Lett* **36**, 168–180 (1977)
8 Cortini, M *Bull. volca.* **44–4**, 711–722 (1981)
9 Vollmer, R. *Geochim cosmochim. Acta* **40**, 283–295 (1976)
10 Capaldi, G, Cortini, M & Pece, R. *Isotope Geosci.* **1**, 39–55 (1983)
11 Capaldi, G & Pece, R. *J volcan geotherm. Res* **11**, 367–372 (1981).
12 Capaldi, G *et al. J geophys Res* **81**, 350–358 (1976)
13 Barberi, F, Gasparini, P, Innocenti, F & Villari, L *J geophys Res* **78**, 5221–5232 (1973)
14 Vollmer, R., Johnston, K, Ghiara, M R., Lirer, L & Munno, R *J volcan geotherm. Res* **11**, 317–327 (1981)
15 Di Girolamo, P *Bull volcan* **41–3**, 1–22 (1978)
16 Stacey, J S & Kramers, J D *Earth planet Sci. Lett* **26**, 207–221
17 Sun, S S *Phil. Trans R. Soc.* **A297**, 409–445 (1980)

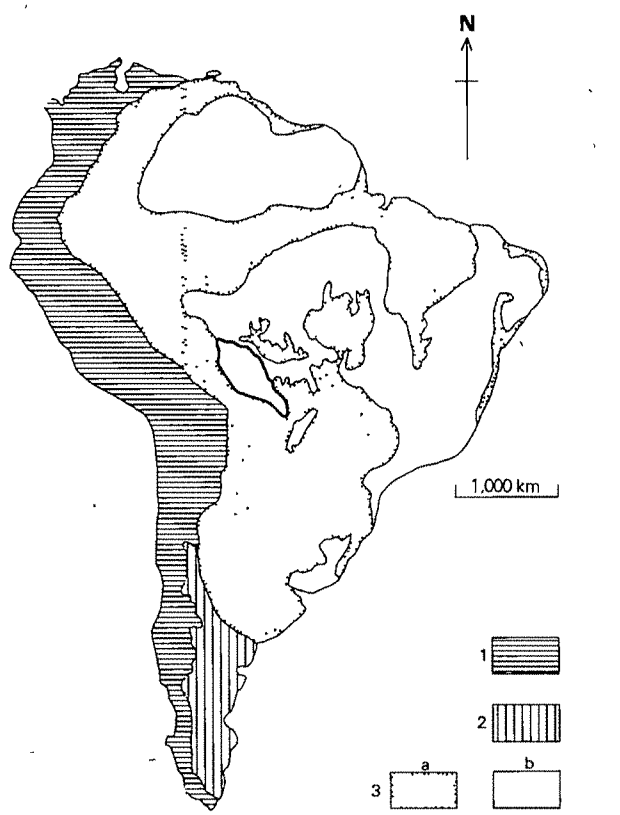


Fig. 1 The major geotectonic regions of South America[11] showing: (1) Andean Chain; (2) Patagonia Platform; (3) South America Platform—a, Phanerozoic cover; b, Brazilian Shield. The eastern Bolivia mapping area is in bold outline.

Andean-trending mobile belts in the Brazilian Shield

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Much of the Phanerozoic Andean mountain chain is floored by Precambrian basement[1–3], in places as old as 2,000 Myr[4,5], but relations between these rocks and those of the Brazilian Shield have long been obscure, mainly because of the intervening Cenozoic sedimentary cover. We describe here recent work over the Bolivian sector of the Brazilian Shield (Fig. 1) which shows a pattern of Proterozoic mobile belts approximately parallel with the Andean chain to the west. When the trends of these Bolivian belts are projected towards the Andes under the Cenozoic cover, their ages broadly accord with those of the Andean basement. Our interpretation is that the two domains are linked in the form of a chain of five Andean-trending mobile belts with a combined width of ~1,000 km, becoming progressively younger to the west with ages ranging from 1,300 Myr to the present.

An Anglo-Bolivian mineral exploration project has completed recently the reconnaissance mapping of the Bolivian Precambrian shield (refs 6–8). The outcrop pattern is determined largely by the San Ignacio Orogeny[7], a medium- to high-grade tectono-metamorphic event, which established a complex of granulites, gneisses and metasedimentary schist belts as well as extensive syn- to post-tectonic granites dated by Rb/Sr at 1,350–1,300 Myr BP (ref. 6 and M.L. *et al.*, in preparation) (Fig. 2). The protolithic rocks of the granulites are considered to be a thick sedimentary sequence and a calc-alkaline plutonic suite, which Rb/Sr studies[6] indicate to be at least 2,000 Myr old, the age of the Trans-Amazonian Orogeny[9]. The semipelitic gneisses, in part higher-grade equivalents of the schist belt, are composed of a metamorphosed sequence of pelites and psammites, with minor volcanics, mafic igneous sills, ironstones and graphitic and calcareous sediments[7]. Palaeocurrent directions preserved in low-grade belts indicate a northerly provenance, from the direction of the present Amazonic Craton[6] (Fig. 3). The schist belt sequence is thought to belong to a San Ignacio Cycle of deposition following the Trans-Amazonian Orogeny[6].

The trend of the San Ignacio Belt is defined by the upright third phase of folding (Fig. 3), which was accompanied by penetrative deformation and the generation of the syntectonic granites[6]. This event folded the granulite-gneiss-schist metamorphic sequence established during the earlier sub-horizontal tectonic phase of the Orogeny[6].

It is now proposed that the limit of the San Ignacio Orogeny in Brazil is the Ciriquiqui arc of mafic/ultramafic intrusives[10] (Fig. 3) which follows the San Ignacio trend. North-east of this line there are relatively undeformed supracrustal sequences, the Beneficiente Group and the Roosevelt Volcanic Formation (Fig. 2), dated at 1,600 Myr BP[10], which rest unconformably on older Xingu Complex basement. These supracrustal units belong[11] to a 1,700–1,400-Myr BP phase of faulting, volcanism, plutonism and sedimentation. They are cut by the anorogenic 1,300-Myr BP Providencia Granites[10] (Fig. 2), accompanied by a widespread K/Ar resetting of older dates, the Madeira event[11], a cratonic reactivation which we relate to the effects of the adjacent San Ignacio Orogeny. Other Brazilian geologists[12,13] classify these same 1,700–1,400-Myr BP rocks as part of the Rio Negro-Juruena Belt, interpreted[14] as a magmatic arc accreted to an older Amazonic nucleus to the north-east.

When the San Ignacio Belt is projected along its west north-west to north-west course under the Cenozoic cover (Fig. 4), it can be linked to similar gneisses and granulites found as massifs in the Columbian Andes which give Rb/Sr and U/Pb dates of 1,300–1,200 Myr BP[15]. Rocks of the Brazilian Shield to the east of this area have undergone the Parguazan reworking of

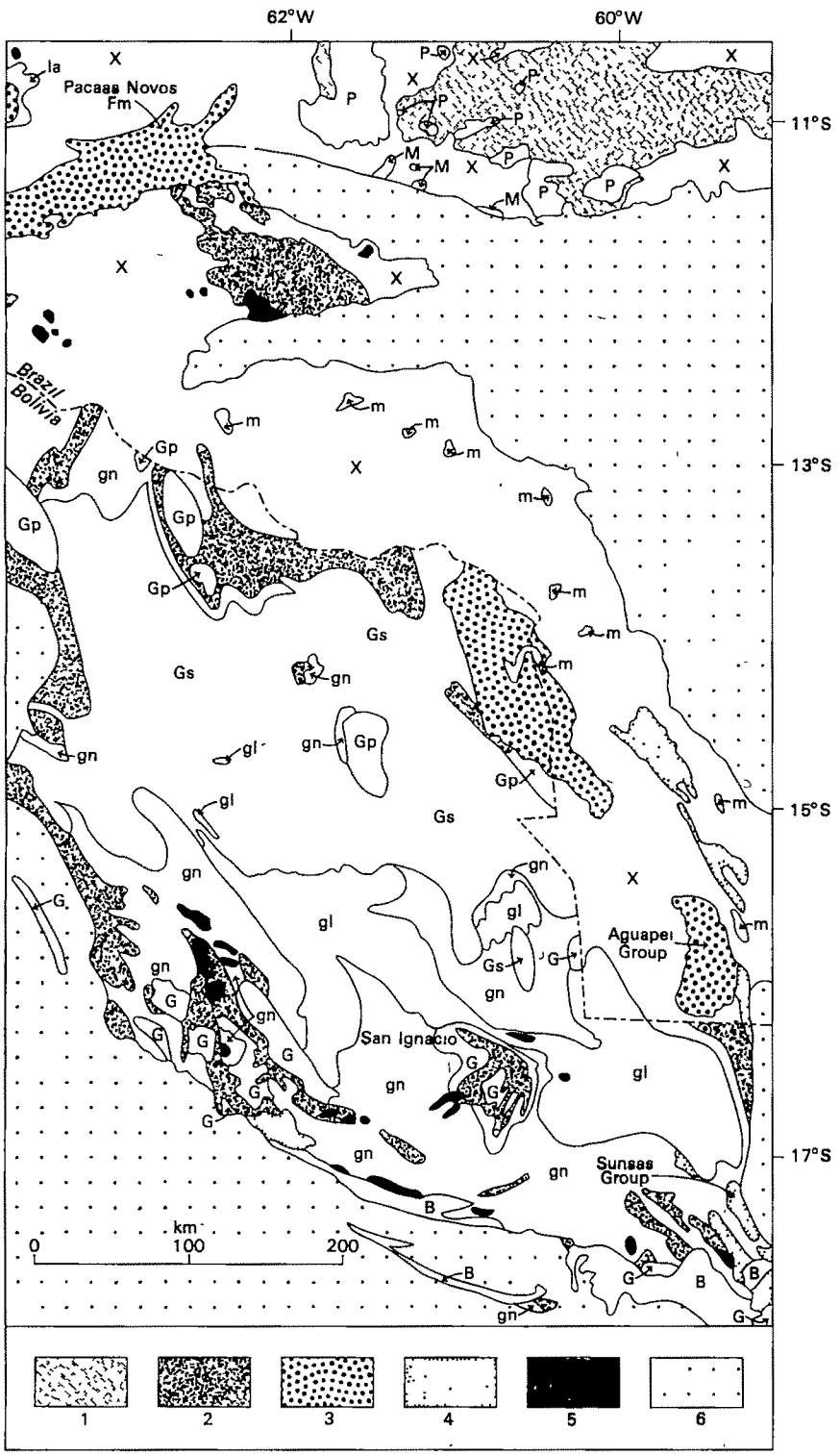


Fig. 2 Precambrian geology of eastern Bolivia[6,8] and adjacent Brazil[26]; tectonic contacts and Mesozoic igneous units omitted. The protolithic rocks of the following are all ≥1,400 Myr BP: gl ≡ granulites; gn ≡ gneisses; X ≡ Xingu Cx; (1) platform Beneficiente Group and Roosevelt Volcanic Formation; (2) San Ignacio schist belts. Of ~1,300 Myr BP: M ≡ mafic/ultramafic intrusives; P ≡ anorogenic Providencia Granites; G ≡ orogenic granites (Gs, syntectonic; Gp, post-tectonic). Of 1,300–1,000 Myr BP: (3) platform Sunsas Group; (4) mobilized Sunsas Group; la ≡ basalt lavas. Of ~1,000 Myr BP: m ≡ mafic/ultramafic intrusives; (5) Rondonia/Sunsas granites. B ≡ Brasiliano Cycle sediments (~600 Myr BP); (6) Phanerozoic cover.

1,500 Myr BP[16] and also exhibit the ~1,300-Myr BP K/Ar resetting known locally as the Nickerie event[15].

Traced southwards through the Bolivian shield area the fold trends of the San Ignacio Belt swing from east south-east through south-east to south (Fig. 3). This is a primary swing and unrelated to secondary deformation of San Ignacio Orogeny age[6]; it can even be identified across the later Sunsas shear zones[7] (Fig. 3). Further south, the San Ignacio Belt is projected west of the 1,700-Myr BP Rio Apa Complex[17] (Fig. 4), but its continuation through Paraguay is conjectural.

The 1,300-Myr BP San Ignacio Orogeny was followed by a period of clastic dominantly-shallow-water deposition, forming the Sunsas and Vibosi Groups in Bolivia[6,7] and their Brazilian correlatives, the Aguapei Group and Pacaas Novos Formation[10], which are locally intercalated with basaltic lavas (Fig. 2). Thicknesses are in the range of 800–5,000 m and there is always a marked basal unconformity. Palaeocurrent directions indicate a northerly source and there are similarities in volcaniclastic suites with those of the older San Ignacio schist belt psammites. Outcrops of this regional unit occur as prominent ridges, representing metamorphosed synformal infolds, or as mesas of flat-lying relatively-undisturbed strata. These can be used as lithostratigraphical indicators in elucidating the tectono-metamorphic history of the Sunsas Orogeny, dated by Rb/Sr and K/Ar at 1,000 Myr BP[6]. Two mobile belts can be recognized separated by a cratonic zone of undisturbed basement capped by Sunsas Group mesas (Figs 2 and 3). The west north-west trending Sunsas Belt is bounded and segmented by curvilinear

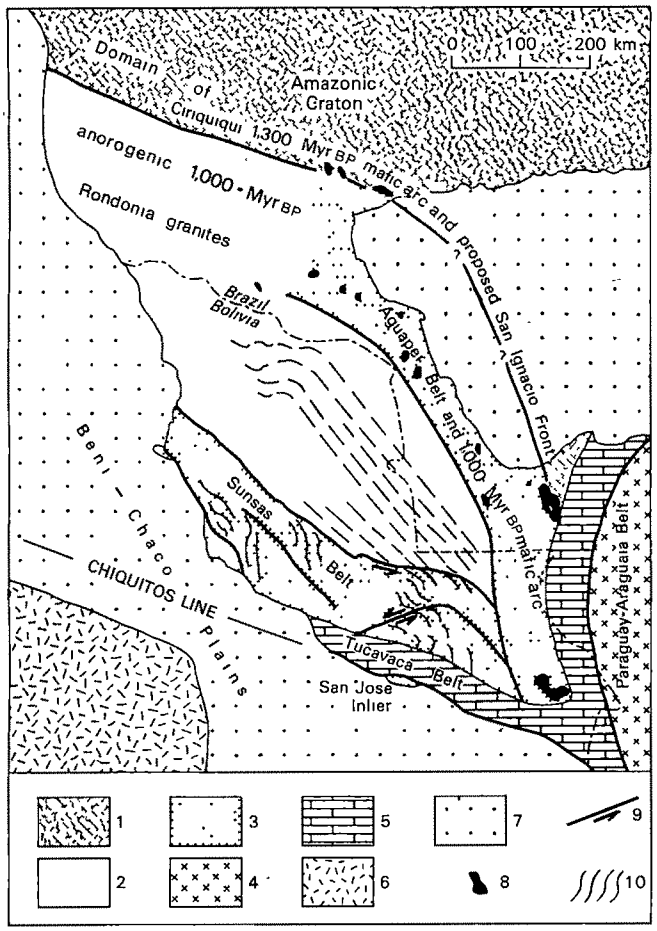


Fig. 3 Geotectonic elements of eastern Bolivia and environs. (1) Cratonic nucleus (≥1,400 Myr BP), (2) San Ignacio Mobile Belt (1,300 Myr BP; (3) 1,000-Myr BP mobile belts; (4) Brasiliano mobile belt (600–500 Myr BP); (5) Brasiliano platform cover; (6) Andean chain; (7) main areas of Phanerozoic platform cover; (8) mafic/ultramafic intrusions; (9) proposed shear zone or tectonic front, arrow indicates sense of horizontal movement; (10) San Ignacio fold trends.

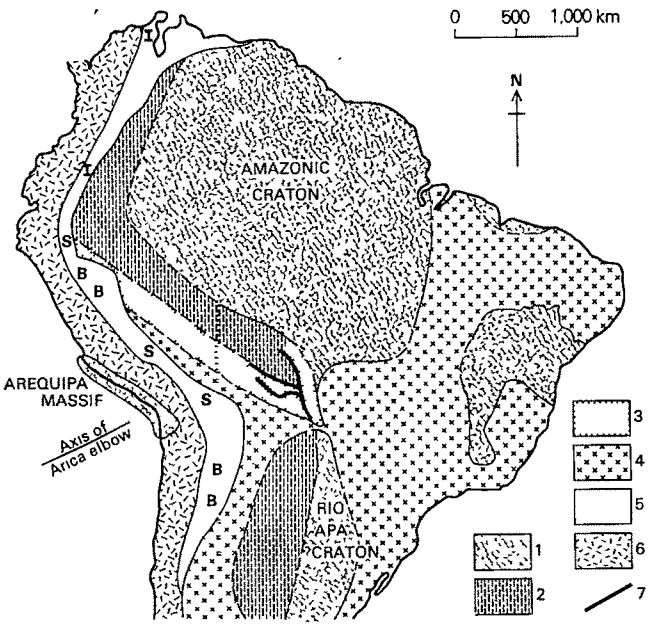


Fig. 4 Present interpretation of main South American geotectonic units; dashed lines indicate projection below South American Platform cover[11]. (1) Cratonic nucleii (≥1,400 Myr BP); (2) San Ignacio Mobile Belt (1,300 Myr BP); (3) Sunsas and Aguapei Mobile Belts (1,000 Myr BP); (4) Brasiliano Mobile Belts (600–500 Myr BP); (5) Eastern Cordillera (Palaeozoic); (6) Western Cordillera (Mesozoic–Cenozoic); (7) 1,000-Myr BP shear zone. I, S and B refer respectively to San Ignacio, Sunsas and Brasiliano orogenic ages of sub-Andean basement rocks. Partially reworked tracts of older rocks within Brasiliano belt terrain[11] are omitted.

sinistral shear zones which enclose sectors marked by coeval upright folds, metamorphism to garnet-grade and a penetrative deformation which cuts earlier recumbent 'thin-skin' folds in the Sunsas Group. Syn- and post-tectonic granites were developed accompanied by tin- and tantalum-bearing pegmatites[7,8]. The Aguapei Belt (Fig. 3), in contrast, is a domain of faulting, folding and mafic-ultramafic intrusives. It seems to deflect the Sunsas shears in a dextral sense (Fig. 3) and follow the trend of the older San Ignacio Belt.

Tracing the Aguapei Belt to the north-west, the folding dies out and the mafic/ultramafic intrusives give way to the domain of the 1,000-Myr BP anorogenic Rondonia granites[18] (Fig. 3). When the Sunsas Belt is projected west north-west to the Andes (Fig. 4), however, it lines up with probable 1,000-Myr BP basement rocks in Ecuador[3]. Sunsas Orogeny-age rocks also floor sectors of the Andes in Bolivia[19] and Peru[1] and may represent older nuclei of an extensive Sunsas Belt within the proposed Brasiliano Belt (Fig. 4).

The Upper Proterozoic–Eocambrian Brasiliano (Pan-African) Cycle of the eastern Bolivian shield is restricted to the western fringe of the eugeosynclinal Paraguay–Araguaia Belt[11] and the west north-west-trending Tucavaca Belt of miogeosynclinal rocks[7] (Fig. 3), bordered to the south by a tectonic front giving reset K/Ar dates of 520 Myr BP[6] in the San José inlier (Fig. 3). The Brasiliano sequences extend under the Phanerozoic cover further south to link up[20] with the Andean-trending 600–500-Myr BP Brasiliano belt recognized in the Sierra Pampeanas of Argentina[21] and other sectors of the sub-Andean basement[1,3] (Fig. 4). The Tucavaca front was reactivated during the Phanerozoic as the Chiquitos Line[22] (Fig. 3) controlling the distribution of Palaeozoic, Cretaceous and Cenozoic sedimentary basins[6].

The Phanerozoic Andean chain can be divided into the Palaeozoic fold belt of the Eastern Cordillera and the Mesozoic–Cenozoic belt of Andean orogeny of the Western Cordillera[1–3]. Figures 3 and 4 illustrate the parallelism of the Bolivian San Ignacio and Aguapei Belts with the Arica 'elbow': the major swing from north to west north-west in the trends of both the eastern and western Cordilleras parallel to the continental margin. There is also a parallelism between the west north-west-trending segment of the elbow and the Sunsas and Tucavaca Belts. The projected continuation of the belts away from the Bolivian outcrop (Fig. 4) accords broadly with the known Precambrian ages of the Andean basement. Thus, an enlarged Andean chain, ~1,000 km wide, can be proposed, consisting of five mobile belts which become progressively younger to the west towards the present continental margin. There is the possibility of an even older sixth belt within the Amazonic Craton (Fig. 4), in the form of the Parguazan reworking of 1,500 Myr BP[16] representing the north-west sector of the Rio Negro–Juruena Belt[13]. Whether such apparent continental growth layers relate to accretion of new crust or reworking of old must depend on the status of the 2,000-Myr BP Arequipa Massif[4] (Fig. 4). Palaeomagnetic studies[5] suggest it is not an allochthonous post-Jurassic microplate but an integral part of the American plate, continuous beneath the Andes with the Brazilian Shield and possibly extending further west before the onset of Mesozoic closure/subduction[1]. If its position with respect to the Amazonic Craton has not changed since 2,000 Myr BP, then all five belts are probably ensialic, with continental margin subduction beginning in the Phanerozoic. However, if the Massif arrived after that time, then the San Ignacio Belt could have developed along a pre-Pacific oceanic suture. In either case, it appears that the trend of the

1,300-Myr BP San Ignacio Belt has controlled those of subsequent orogenies as well as the present shape of the continental margin, suggesting a longstanding tectonic regime established within the continent and perhaps related to the formation of the Pacific Ocean.

The identification of Proterozoic elements in an enlarged Andean chain would augment, at least locally, the number of peripheral mobile belts which young towards the southern continental margin of Gondwanaland[23], with implications regarding the mechanisms of formation, such as accretion or reworking[24], of the Brasiliano (Pan-African) belts Traced to North America, the 1,300-Myr BP San Ignacio Belt may deviate from the circum-Pacific chain to link up[15] with the Grenville Belt of the North Atlantic chain[25].

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1 Dalmayrac, B, Laubacher, G & Marocco, R *Caracteres généraux de l'Évolution géologique des Andes peruviennes* (Office de la Recherche Scientifique et Technique Outre-Mer, Paris, 1980)
2 Cobbing, E J in *Ocean Basins and Margins* (Plenum, New York, in the press)
3 Zeil, W *The Andes, A Geological Review* (Gebruder Borntraeger, Berlin, 1979)
4 Cobbing, E, Ozard, J M & Snelling, N J *Bull geol Soc Am* **88**, 9-17 (1977)
5 Shackleton, R M, Ries, A C, Coward, M P & Cobbold, P R *J geol Soc.* **136**, 195-214 (1979)
6 *Unpublished Proyecto Precambrico final reports* Open file, GEOBOL (Bolivia) and BGS (Keyworth, Nottingham UK)
7 Litherland, M & Bloomfield, K *Precambrian Res* **15**, 157-179 (1981)
8 Berrangé, J P *Episodes* 4, 3-8 (1982)
9 Cordani, U G, Amaral, G & Kawashita K *Geol Rdsch* **63**, 9-17 (1973)
10 Leal, J W L *et al Projecto Radambrasil* **16** (Departamento Nacional da Produção Mineral, Rio de Janeiro, Brazil, 1978)
11 De Almeida, F F M, Hasui, Y, de Brito Neves, B B & Fuck, R A *Earth Sci Rev* **17**, 1-29 (1981)
12 Cordani, U G *et al. II Congr Geol Chileno, Arica*, **1**, 137-148 (1979)
13 Cordani, U G & de Brito Neves, B B *Rev Bras Geoc* **12**, 78-88 (1982)
14 Tassinari, C C G *Anais II Symp Amaz Manaus*, 439-446 (1984)
15 Kroonenberg, S B *Geologie Mijnb* **61**, 325-333 (1982)
16 Priem, H N A *et al Geologie Mijnb* **61**, 229-242 (1982)
17 Del'Arco, J O *et al Simp Geologia do Centro-Oeste Soc Bras Geol*, 154-176 (1981)
18 Priem, H N A *et al Bull geol Soc Am* **82**, 1095-1102 (1971)
19 Lehmann, B *Geol Rdsch* **67**, 270-278 (1978)
20 O'Connor, E A & Walde, D H G *Abstr 9th Geowissench Lateinamerika Koll.* (Marburg, 1984)
21 Miller, H *J geol Soc.* **141**, 885-892 (1984).
22 Gansser, A *J geol Soc* **129**, 93-131 (1973)
23 Windley, B F *The Evolving Continents* (Wiley, New York, 1977)
24 Kroner, A in *Precambrian Plate Tectonics*, 57-90 (Elsevier, Amsterdam, 1981)
25 Williams, H *Can J earth Sci* **21**, 887-901 (1984)
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Oceanic solitons, nutrient pulses and phytoplankton growth

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Phytoplankton growth in the sea is influenced by internal wave activity both through vertical displacements of the pycnocline, which cause fluctuations in the light environment for photosynthesis, particularly in the subsurface chlorophyll maximum[1,2], and through mixing processes associated with energy dissipation[3]. In shallow continental-shelf waters, mixing by internal waves can lead to pulses of nutrients in the nutrient-depleted surface layers[4]. In the deep oceans, increased nutrient fluxes resulting from internal wave mixing have also been postulated to explain anomalously high primary production values[5], but this phenomenon has not been observed directly. Here we present the first evidence from a deep (4,000 m) oceanic environment for nutrient mixing by large soliton-like internal waves[6-9], generated more than a day before at the shelf edge. The observations were made in the Bay of Biscay (Fig. 1*a*), where the level of nitrate is considered to be a major factor regulating the standing stock and productivity of phytoplankton in surface water during the summer months[10,11], as in other areas of the eastern North Atlantic Ocean[12].

Earlier studies along the edge of the north-west European shelf showed that internal waves are generated in discrete groups or packets close to the 200-m contour and propagate on-shelf with on-shelf tidal streaming[13]. These are best developed at spring tides[14]. Synthetic Aperture Radar (SAR) images from the Seasat satellite indicate that internal waves also travel outwards from the shelf-break into the deeper waters of the Bay of Biscay[13]. Our investigations during large spring tides at the end of August 1984, confirmed the existence of these oceanic solitons, which were detected acoustically (Fig. 1*b*) as they propagated normally and away from (~210°) the shelf edge. Each packet of two to six large waves (followed by a train of smaller waves) was formed on a single tidal excursion. The peak-to-trough amplitude of these larger waves was 50–80 m, with a wavelength of 1–1.5 km and a phase speed of ~1.1 m s^{-1}. With calm winds (< 5 m s^{-1}), similar characteristics were found at distances of up to 240 km from the shelf-break, corresponding to a travel time of more than two days Throughout this time, the solitons could be seen visually and by radar as parallel bands of rough and smooth water of up to 10 km in length with associated lines of floating debris and scum. Records from a towed, undulating CTD-fluorometer system[15,16] also show the presence of large internal waves (Fig. 1*c*, *d*). Within the limits of spatial resolution set by the sampling strategy, the variations in the depth of the subsurface chlorophyll maximum[17] were consistent with the temperature (density) structure. These variations produce large fluctuations in ambient light conditions for the plant cells; for example, given a typical value for the extinction coefficient, k, of 0.08 m^{-1}, the light intensity at the level of the chlorophyll maximum in Fig. 1*d* is estimated to range from ~6.0 to 0.03% of surface values.

Although the echo traces showed no overturning[18] on a vertical scale of the internal wave amplitude, expendable bathythermograph (XBT) temperature profiles through individual waves exhibited occasional temperature inversions (Fig. 2) These temperature profiles are representative of the density profiles, as changes in salinity with respect to depth were both small (~0.05‰ from the surface down to 200 m) and destabilizing. The inversions cannot be interpreted in terms of interleaving between adjacent water masses, as horizontal salinity gradients above and below the pycnocline were also very weak (< 0.01‰ over 10 km). The most probable explanation, therefore, is active vertical mixing, at low Richardson Number conditions, due to the passage of the internal wave[19]. Areas of surface roughness, ~5 m across, in smooth water near the internal wave crests (Fig 3) indicated small-scale turbulent events within the surface mixed layer. Local increases in surface nutrients (NO_3, NO_2, Si; Fig. 4*a*) were also detected as the wave packet passed the ship, similar to nutrient patches observed on the shelf in 1983 (P.M.H., R.D.P. and G.T.M., unpublished data), in association with a large amplitude internal wave packet propagating on the continental shelf[14]. The smoothing effects in the nutrient sampling system are such that the spatial extent of the nutrient patch (Fig. 4*a*) is unresolved and, as far as the instrumentation is concerned, is equivalent to a point source. The ephemeral nature of nutrient changes associated with internal waves was also a feature of measurements from the Southern Californian Bight[4].

All crossings of the shelf break between 20 August and the period of strongest tides on 29–30 August had shown characteristic surface cooling and enhanced chlorophyll fluorescence[17] but

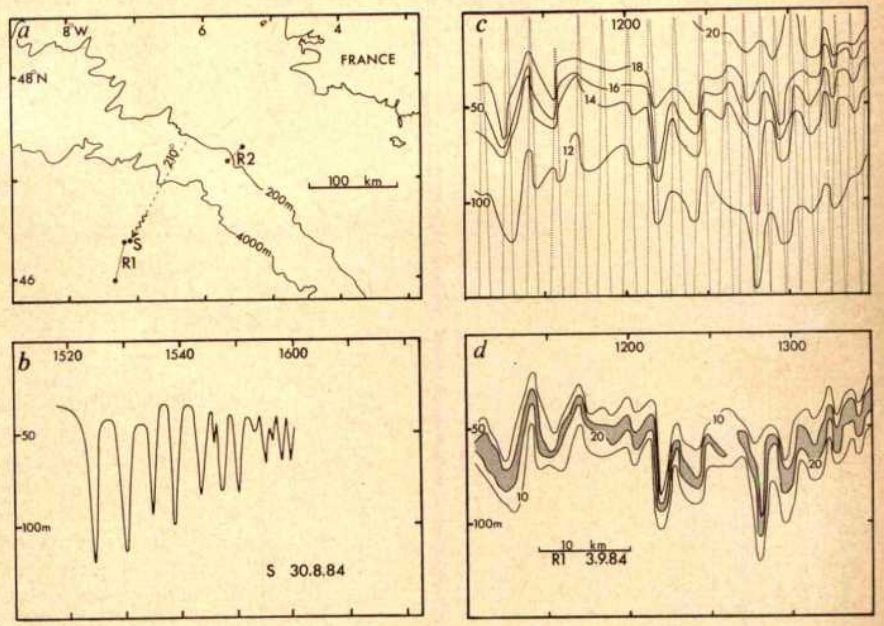


Fig. 1 *a*, Bathymetric map of the northern Bay of Biscay. The wavy arrow indicates the direction of soliton propagation and points to the location (S) of the observations shown in Figs 1*b*, 2, 3 and 4*a*. R1 and R2 are the ship's tracks for the hydrographic records illustrated respectively in Figs 1*c*,*d* and 4*b*. *b*, Depth of the scattering layer (corresponding approximately to the 14 °C isotherm) against time (GMT) at a position ~140 km from the shelf-break (see *a*) as the RRS *Frederick Russell* steamed 020 °T at 5 knots on 30 August 1984. The internal waves are propagating from right to left (in the figure) in a direction 210° as determined from the ship's radar. A XBT section and temperature profiles through the leading wave of the soliton packet are shown in Fig. 2. A surface nutrient pulse was observed at 15:38 GMT (Fig. 4*a*). Water depth is 4,600 m. *c*,*d*, Undulator records for 11:00–13:30 GMT 3 September 1984, showing the distributions of temperature (°C) and chlorophyll fluorescence (arbitrary units) against depth (m). The fluorescence contours correspond to chlorophyll-*a* concentrations of about 0.4 and 0.8 mg m^{-3}. The dotted lines in *c* indicate the path of the towed undulator system.

uniformly low (<0.2-μM) surface nitrate values. On 1–2 September, however, nitrate concentrations up to 1.5 μM were found at a location close to the 200-m contour (Fig. 4*b*), with values >1 μM over a distance of ~7 km normal to the shelf edge corresponding to a band of cold water. Throughout this period winds were weak (<5 m s^{-1}) and variable in direction, indicating that the increase in surface nutrients close to the shelf-break resulted from the cumulative and continuous effects of strong tidal streams and internal wave activity rather than from wind-induced upwelling[20].

During the summer months, the coupled effects of tides and wind promote vertical mixing within the relatively shallow (<200-m) water column on the continental shelf. By contrast, in the deep water environment of the Bay of Biscay, bottom mixing by tides is irrelevant and the effect of wind-mixing above a relatively deep pycnocline (~50 m, Fig. 1*c*) is much reduced so that vertical nutrient fluxes across the seasonal pycnocline are relatively weak[10]. Furthermore, the subsurface chlorophyll maximum acts as a sink for nutrients mixed upwards from below the pycnocline[21]. Under these conditions phytoplankton growth in the surface waters tends to be restricted by the rate of nutrient supply from biological regeneration processes[11,22,23]. The occurrence of the observed density inversions, surface nutrient patches and surface turbulence features in association with solitons indicates that mixing by internal waves is an important physical mechanism for maintaining upward fluxes of nutrients (another mechanism may be mesoscale eddies[24]) as has been suggested[5] for the North Pacific gyre. This is likely to be particularly significant at the level of the subsurface chlorophyll maximum in the pycnocline, where mixing by internal waves will produce more frequent nutrient pulses than are encountered at the sea surface.

Mixing by spring-tide solitons will be repeated at fortnightly intervals throughout the summer and influence phytoplankton

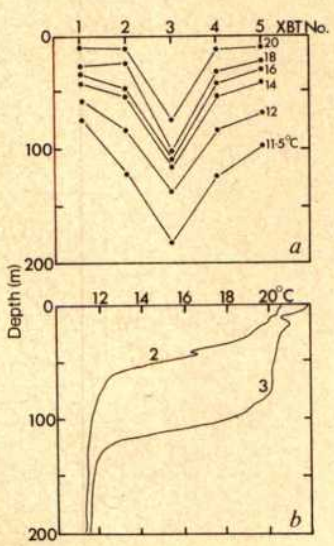


Fig. 2 *a*, Expendable bathythermograph (XBT) section across the first wave in the soliton packet illustrated in Fig. 1*b*. *b*, XBT profiles through the leading wave of the soliton packet showing temperature inversions.

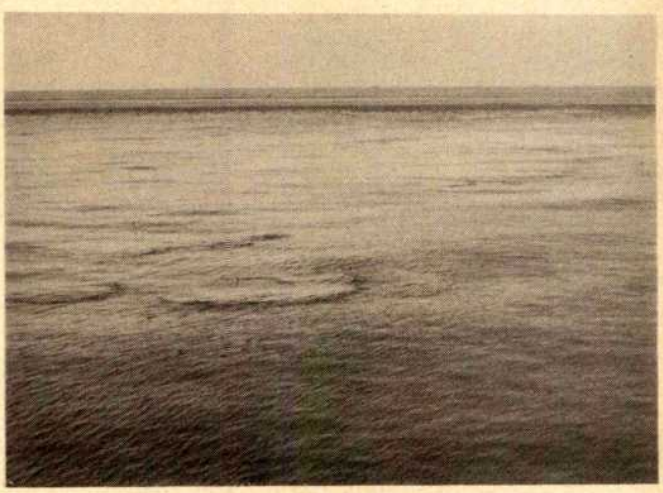


Fig. 3 Sea surface state at position S (Fig. 1*a*) within soliton packet (Fig. 1*b*) at 15:00 GMT, 30 August 1984. Rough water, corresponding to the internal wave troughs, appears as dark bands towards the horizon. Surface layer turbulence is shown as disturbed patches, ~5 m across, in the foreground. Water depth is 4,600 m.

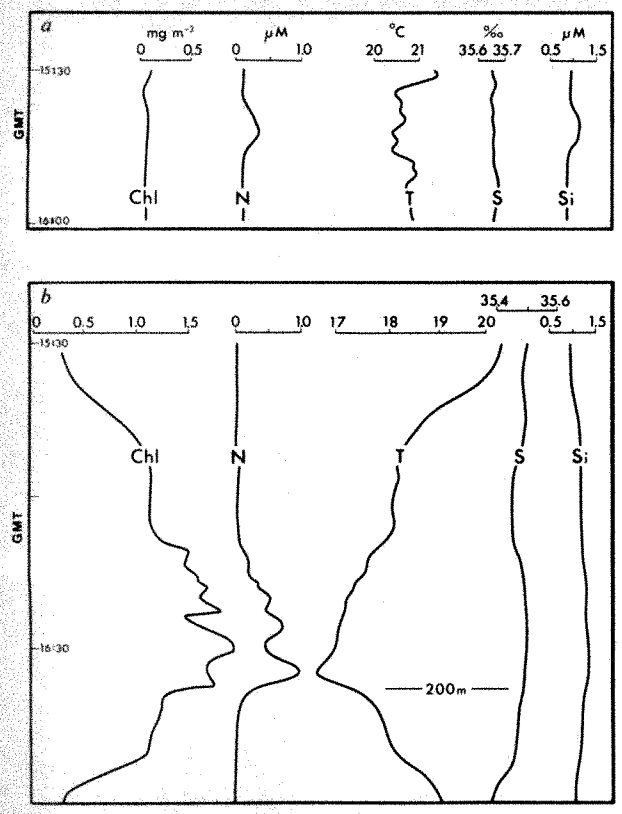


Fig. 4 Continuous surface (3.5-m) records of chlorophyll-*a* (Chl. mg m^{-3}) nitrate (N, μM), temperature (*T*, °C), salinity (*S*, ‰) and silicate (Si, μM) for *a*, position S, 15:30–16:00 GMT, 30 August 1984, and *b*, track R2, 15:30–17:00 GMT, 2 September 1984, crossing the shelf-break at 16:37 GMT. The ship's positions are shown in Fig. 1*a* and in both cases the ship was heading in a southwesterly direction.

growth in the thermocline at great distances from the shelf-break. The horizontal extent of this effect will vary because of interaction with wind mixing and changes in the depth of the thermocline resulting from the generation of near inertial oscillations[25]. At times of stronger winds, it is anticipated that mixing events due to instabilities associated with the passage of internal waves will occur more frequently and the nutrient pulses will then be more concentrated in the slope region.

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1. Haury, L. R., Wiebe, P. H., Orr, M. H. & Briscoe, M. G. *J. mar. Res.* **41**, 65–112 (1983).
2. Kahru, M. *Mar. Ecol. Prog. Ser.* **10**, 111–117 (1983).
3. Garrett, C. & Munk, W. *Deep-Sea Res.* **19**, 823–832 (1972).
4. Cullen, J. J., Stewart, E., Renger, E., Eppley, R. W. & Winant, C. D. *J. mar. Res.* **41**, 239–262 (1983).
5. McGowan, J. A. & Hayward, T. L. *Deep-Sea Res.* **25**, 771–793 (1978).
6. Zabusky, N. J. & Krustal, M. D. *Phys. Rev. Lett.* **15**, 240–243 (1965).
7. Osborne, A. R. & Burch, T. L. *Science* **208**, 451–460 (1980).
8. Sandstrom, H. & Elliott, J. A. *J. geophys. Res.* **89**, 6415–6426 (1984).
9. Briscoe, M. G. *Nature* **312**, 15 (1984).
10. Tréguer, P., Le Corre, P. & Grall, J. R. *Deep-Sea Res.* **26A**, 1121–1152 (1979).
11. Eppley, R. W., Renger, E. H. & Harrison, W. G. *Limnol. Oceanogr.* **24**, 483–494 (1979).
12. Williams, R. & Robinson, G. A. *Bull. Mar. Ecol.* **7**, 115–121 (1973).
13. Pingree, R. D. & Mardell, G. T. *Phil. Trans. R. Soc.* **A302**, 663–682 (1981).
14. Pingree, R. D. & Mardell, G. T. *Prog. Oceanogr.* **14**, 431–441 (1985).
15. Collins, D. S., Pollard, R. T. & Suchen, P. V. *Inst. Oceanogr. Sci. Rep.* **148**, 1–75 (1983).
16. Fasham, M. J. R., Pugh, P. R., Griffiths, D. & Wheaton, J. E. G. *Proc. Inst. Electr. Radio Engng Lond.*, 49–58 (1981).
17. Pingree, R. D., Mardell, G. T., Holligan, P. M., Griffiths, D. K. & Smithers, J. *Contin. Shelf Res.* **1**, 99–116 (1982).
18. Haury, L. R., Briscoe, M. G. & Orr, M. H. *Nature* **278**, 312–317 (1979).
19. Woods, J. D. & Wiley, R. L. *Deep-Sea Res.* **19**, 87–121 (1977).
20. Heaps, N. S. *Oceanol. Acta* **3**, 449–454 (1980).
21. Pingree, R. D., Holligan, P. M. & Head, R. N. *Nature* **265**, 266–269 (1977).
22. King, F. D. & Devol, A. H. *Limnol. Oceanogr.* **24**, 645–651 (1979).
23. Holligan, P. M., Williams, P. J. LeB., Purdie, D. & Harris, R. P. *Mar. Ecol. Prog. Ser.* **17**, 201–213 (1984).
24. Madelain, F. & Kerut, E. G. *Oceanol. Acta* **1**, 159–168 (1978).
25. Mazé, R. thesis, Univ. Bretagne Occidentale (1983).

Lake acidification in Galloway: a palaeoecological test of competing hypotheses

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Possible causes of lake acidification in Britain include acid precipitation, heathland regeneration, afforestation and post-glacial natural acidification (long-term change). In Galloway, south-west Scotland, several lakes with non-afforested catchments have been acidified by approximately 1 *p*H unit since about 1840[1,2], which clearly precludes long-term change and afforestation as causes of the present acidity, but does not discriminate between the possible effects of heathland regeneration and acid precipitation; it *could* be argued that a decline in upland farming in this area led to an increase in acid heathland communities capable of promoting lake acidification during this period of time. Here we use pollen analysis to show that there is no evidence for an increase in *Calluna vulgaris*, the most important heathland species, over the past 200 yr or so, and we substantiate the acid precipitation hypothesis by demonstrating substantial increases of the heavy metals Pb, Cu and Zn in the sediment since about AD 1800.

Surface water acidification has been variously ascribed to the effects of acid precipitation[3], land-use change[4], afforestation[5,6] and long-term change[7], the importance of which can be assessed by palaeoecological techniques. We consider the last two of these processes first.

Long-term acidification is well known, having been recognized in Scandinavia[8–11], Britain[12–15] and North America (ref. 16 and J. Ford, unpublished results). In Britain it has been suggested that this process is the cause of contemporary acidity in upland tarns of the English Lake District and that certain lakes were so acidic by 1800 that no further change can have occurred since then[7]. Although this supposition has not yet been examined in the context of the Lake District, it is clear that the present acidity of lakes with granitic catchments in Galloway cannot be explained in this way; diatom analysis indicates that rapid acidification of these lakes has occurred since about 1840, after long periods of stability[1,2].

Recent afforestation, although responsible for accelerated soil erosion[17] and other undesirable ecological changes, is also of little significance as lakes with non-afforested catchments were

Table 1 Physical and chemical characteristics for Loch Enoch

Altitude (m)	493	
Lake area (ha)	50	
Catchment area (ha)	186	
Catchment: lake area	3.7	
Maximum depth (m)	~36	
Temperature (°C)	7.0 (4.4)	
Conductivity (μS_{18} cm^{-1})	30.0 (8.0)	37*
*p*H (range)	4.4–4.7	4.5*
H^+ (μeq l^{-1})	28.9 (6.8)	28
Na^+ (μeq l^{-1})	58.3 (24.4)	126
K^+ (μeq l^{-1})	6.7 (4.6)	6
Ca^{2+} (μeq l^{-1})	10.5 (3.5)	21
Mg^{2+} (μeq l^{-1})	16.5 (7.2)	37
Al (μeq l^{-1})	10.0 (4.4)	16
Cl^- (μeq l^{-1})	100.9 (18.0)	146
SO_4^{2-} (μeq l^{-1})	44.5 (12.9)	89

Numbers in parentheses indicate standard deviations for the mean values calculated from eight measurements made between November 1981 and November 1982. The catchment area excludes lake area.
* Data in the last column are from ref. 33.

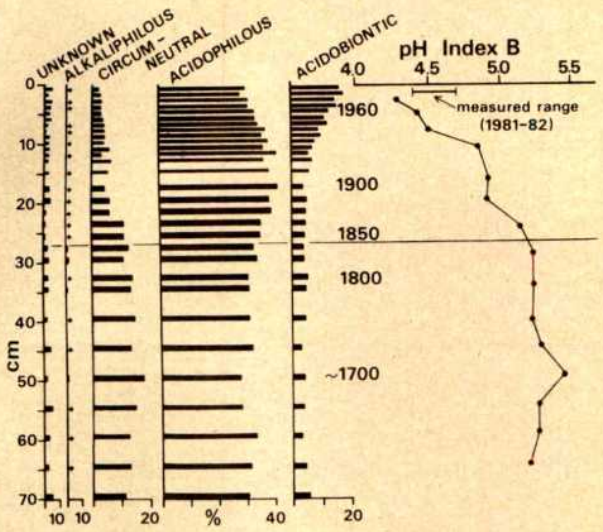


Fig. 1 Summary diatom diagram from Loch Enoch showing changes in the proportions of the various *p*H groups through time. Dominant taxa are as follows: (1) circumneutral, *Anomoeoneis vitrea* (Grun.) Ross; (2) acidophilous, *Eunotia veneris* (Kütz). O. Müll., *E. alpina* (Naeg.) Hust., *Tabellaria flocculosa* (Roth.) Kütz., *Frustulia rhomboides* v. *saxonica* (Rabh.) de Toni; (3) acidobiontic, *T. quadriseptata* Knudson, *T. binalis* (Ehr.) Grun., *Navicula höfleri* Cholnoky. Dates are based on ^{210}Pb measurements[17], and *p*H is reconstructed using index B[11]. Horizontal line signifies point of change.

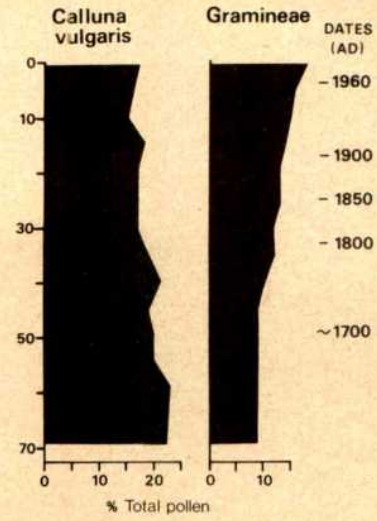


Fig. 2 Proportions of *C. vulgaris* and Gramineae pollen from the Loch Enoch sediment core.

acidified, and those with afforested catchments began to be acidified, in the late nineteenth and early twentieth century, well before the beginning of afforestation[1].

A hypothesis involving change in land use has been proposed to explain recent lake acidification in Norway[4,18]. It maintains that acidity generated by soil processes is substantially greater than that received from atmospheric deposition and that an increase in the formation of acid humus in soils as a result of a decline in upland agriculture is likely to be of more significance than an increase in the acidity of precipitation.

In Galloway it may be expected that a similar decline in grazing would lead to the greater dominance of *C. vulgaris* in particular, and to a regeneration of heathland communities in general[19]. This hypothesis would be rejected if it could be shown that such a land-use change was absent or was inadequate to explain the observed acidification of the lakes concerned, and we would then need to evaluate the acid precipitation hypothesis.

To carry out these tests we have used diatom analysis, pollen analysis and trace metal analysis of a dated lake sediment core from Loch Enoch, a 50-hectare, 36-m deep multi-basin lake situated at an altitude of 493 m on the Merrick in the Rhinns of Kells region of Galloway. The catchment is non-afforested and the vegetation is dominated by *Molinia caerulea* and *C. vulgaris*.

We obtained a sediment core from the centre of the south-east basin of the lake and determined a linear sediment accumulation rate of ~1 mm yr^{-1} using ^{210}Pb analysis[17]. Diatom analysis shows the onset of recent acidification at ~30 cm, dated to about 1840 (Fig. 1). The index B *p*H reconstruction[11] gives a *c.*1840 *p*H of 5.2 and a present *p*H of 4.3, slightly more acidic than the measured range of *p*H values (see Table 1). The strong increase in acidobiontic diatoms registered in the diagram (Fig. 1) include *Tabellaria binalis* (Ehr.) Grun. and *Tabellaria quadriseptata* Knudson, species that typically increase in a similar way at other sites in the region[1,20].

If acidification was caused by a change in land use in the catchment of the lake, in accordance with the above hypothesis, we would expect historical records to show a decline in grazing and consequent regeneration and expansion of heathland on approximately the same timescale. As records of sheep numbers over the past 150 yr are available only for large administrative regions[21], it is more appropriate to test this prediction using pollen analysis of the lake sediment core itself. This has been performed using standard procedures[22] on samples selected at 5-cm intervals from the top of the core. The full pollen diagram shows very little change over the past 200–300 yr, except in the relative proportions of *C. vulgaris* and Gramineae, the taxa of greatest interest (Fig. 2). However, rather than an increase in *C. vulgaris*, as would be expected were the land-use hypothesis valid at these sites, there is a gradual decrease in this species and a reciprocal expansion of Gramineae; similar results have been obtained from the analysis of cores from other lakes in the area both in terms of percentage and flux change (unpublished results). These results are not unexpected as they agree with contemporary observations that all the non-afforested lake catchments in this area are grazed and repeatedly burnt over, a process that in the west of Scotland favours *M. caerulea* and keeps *C. vulgaris* young and palatable for both cattle and sheep[23].

Having rejected three hypotheses for the recent acidification of Loch Enoch, we retain only the acid precipitation hypothesis. This hypothesis would be questionable if we could not show that Loch Enoch and other similar Galloway lakes have received atmospheric pollutants that may be associated with industrial emissions. We therefore analysed the trace metal content of the sediments (Fig. 3).

Total sedimentary Zn, Cu and Pb concentrations were determined by flame atomic absorption after $HF/HNO_3/HClO_4$

Table 2 Background sedimentary concentrations, fluxes and recent fluxes of Zn, Cu and Pb in a sediment core from Loch Enoch

	Zn	Cu	Pb
Background concentration, μg g^{-1}	25.4	6.2	74.6
Standard deviation (less than 32 cm depth, $n = 12$)	7.4	4.6	23.3
Background sedimentary flux, mg m^{-2} yr^{-1} (average value for 32–61 cm)	6.1	1.5	17.8
Recent sedimentary flux, mg m^{-2} yr^{-1} (average value for 0–10 cm)	35.5	8.3	67.0
Anthropogenic sedimentary flux, mg m^{-2} yr^{-1} (recent, background flux)	29.4 (83)	6.8 (82)	49.2 (73)

Calculations were made using trace metal concentrations (Fig. 3) and sediment wet density, dry weight and accumulation rate (ref. 21). Numbers in parentheses indicate % anthropogenic flux of total metal influx.

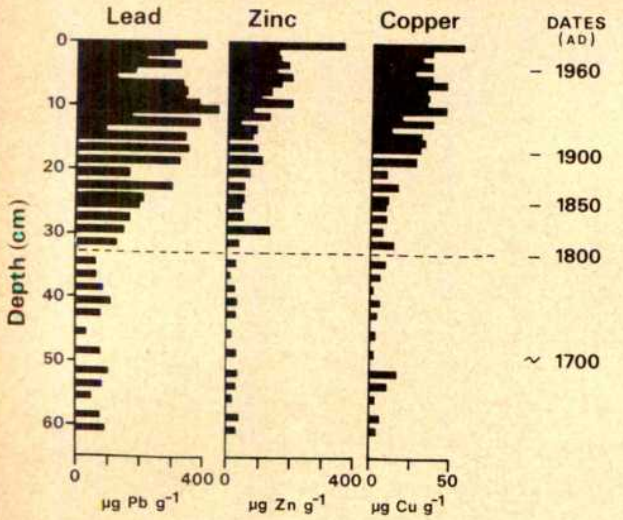


Fig. 3 Concentrations of Pb, Zn and Cu from the Loch Enoch sediment core. Dotted line signifies point of change.

digestion, the precision of the results are, respectively, 4.7, 0.9 and 3.8 μg g^{-1} ($n = 14$). The background concentrations (Table 2) show that there are no geochemical anomalies in the catchment. The slightly lower Cu and Zn and somewhat higher Pb concentrations, compared with the mean for freshwater sediments[24], are a result of the granites in the catchment[25]. The increased concentrations (Fig. 3) and sedimentary fluxes (Table 1) can be accounted for by increased deposition from the atmosphere[26], a situation common in remote and rural lakes[27–29]. It has been shown that atmospheric contamination of lake waters by trace metals is correlated with acidification[30,31], and that contamination of sediments is correlated with sedimentary evidence of acidification[26,27,32]. This latter relationship holds true for Loch Enoch and all these relationships are consistent with expectations derived from the acid precipitation hypothesis.

We cannot prove that an increase in acid deposition was responsible for the acidification of Loch Enoch and similar lakes in Galloway, but we have shown that alternative hypotheses, as presently formulated, are inadequate. Land-use change may play a part in other areas, but at present it is clearly not applicable to the Galloway sites. For this hypothesis to be tested by palaeoecological means, examination of an acidified site in an area of low acid deposition, where heathland regeneration has occurred concurrently, is required.

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1. Flower, R. J. & Battarbee, R. W. *Nature* **305,** 130–133 (1983).
2. Battarbee, R. W. *Phil. Trans. R. Soc.* B**305,** 451–477 (1984).
3. Jensen, K. W. & Snekvik, E. *Ambio* **1,** 223–225 (1972).
4. Rosenquist, I. T. *Sci. tot. Envir.* **10,** 39–49 (1978).
5. Harriman, R. & Morrison, B. R. S. *Hydrobiologia* **88,** 251–263 (1984).
6. Nilsson, S. I., Miller, H. G. & Miller, J. D. *Oikos* **39,** 40–49 (1982).
7. Pennington, W. *A. Rep. Freshwat. biol. Ass.* 28–46 (1984).
8. Quennerstedt, N. *Acta phytogeogr. suec.* **36,** 1–208 (1955).
9. Digerfeldt, G. *Folia limnol. scand.* **16,** 1–104 (1972).
10. Renberg, I. *Early Norrland* **9,** 113–160 (1976).
11. Renberg, I. & Hellberg, T. *Ambio* **11,** 30–33 (1982).
12. Round, F. E. *New Phytol.* **56,** 98–126 (1957).
13. Mackereth, F. J. H. *Proc. R. Soc.* B**161,** 295–309 (1965).
14. Pennington, W., Haworth, E. Y., Bonny, A. P. & Lishman, J. P. *Phil. Trans. R. Soc.* B**264,** 191–294 (1972).
15. Evans, G. H. & Walker, R. *New Phytol.* **78,** 221–236 (1977).
16. Engstrom, D. R. & Wright, H. E. in *Lake Sediments and Environmental History* (eds Haworth, E. Y. & Lund, J. W. G.) (Leicester University Press, 1984).
17. Battarbee, R. W., Appleby, P. G., Odell, K. & Flower, R. J. *Earth Surf. Processes* (in the press).
18. Krug, E. E. & Frink, C. R. *Science* **221,** 520–525 (1983).
19. Gimmingham, C. H. *J. Ecol.* **48,** 455–483 (1960).
20. Flower, R. J. & Battarbee, R. W. *Br. phycol. Bull.* **21,** (in the press).
21. Flower, R. J. & Battarbee, R. W. *Working Pap.* No. 6 (Palaeoecology Research Unit, University College London, 1983).
22. Moore, P. D. & Webb, J. A. *An Illustrated Guide to Pollen Analysis* (Hodder & Stoughton, Dunton Green, 1978).
23. McVean, D. N. & Lockie, J. D. *Ecology and Land Use in Upland Scotland* (Edinburgh University Press, 1969).
24. Forstner, U. *Arch. Hydrobiol.* **50,** 172–191 (1977).
25. Mason, B. & Moore, C. B. *Principles of Geochemistry* (Wiley, New York, 1982).
26. Galloway, J. N., Thornton, J. D., Norton, S. A., Volchok, H. L. & McLean, R. A. N. *Atmos. Envir.* **16,** 1677–1700 (1982).
27. Davis, R. B., Norton, S. A., Brakke, D. F., Berge, F. & Hess, C. T. in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tollan, A.) (SNSF project, Oslo, 1980).
28. Rippey, B., Murphy, R. J. & Kyle, S. W. *Envir. Sci. Technol.* **16,** 23–30 (1982).
29. Wong, H. K. T., Nragu, J. O. & Coker, R. D. *Chem. Geol.* **44,** 187–201 (1984).
30. Henricksen, A. & Wright, R. F. *Wat. Res.* **12,** 101–112 (1978).
31. Wright, R. F. & Henricksen, A. *Limnol. Oceanogr.* **23,** 487–498 (1978).
32. Holdren, G. R., Brunelle, T. M., Matisoff, G. & Wahlen, M. *Nature* **311,** 245–248 (1984).
33. Wright, R. F. & Henricksen, A. *Regional Surv. Lakes Streams Southwestern Scotland* (SNSF project IR 72/80, 1980).

Sediment and pollen evidence for an early to mid-Holocene humid period in the eastern Sahara

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A major problem in the study of Holocene palaeoenvironments of the arid and wind-deflated Sahara is the low preservation potential of sediments from which a record of past climatic change can be established. Several indirect and imprecisely dated pieces of evidence have suggested humid episodes in the Holocene that may have supported more productive ecosystems and denser human populations than today. Searches for reliable proxy data on past climates, however, have failed to yield satisfactory results. We report here the discovery of buried lake muds in north-west Sudan, in the hyperarid core of the Eastern Sahara, which yield sedimentological and palynological data clearly interpretable as recording an early to mid-Holocene humid episode that supported a relatively-deep stratified lake surrounded by tropical savanna woodland vegetation.

The site lies in the Oyo Depression in NW Sudan at 19°16′N and 26°11′E, 510 ± 20 m above sea level (Fig. 1). The depression is surrounded by bedrock and contains Holocene lacustrine sediments buried by variable depths of dune sand[1]. It lies in the hyperarid core of the Sahara, with an estimated mean annual rainfall of ~5 mm (ref. 2), northerly winds with mean daily average velocity of 2–20 km h^{-1}, daily average relative humidity of 20–60% and estimated evaporation rates of ~5 m yr^{-1} (ref. 3). The area is in the zone of absolute desert vegetation whose southern limit is ~500 km south of the site[4] (Fig. 1). Vegetation is confined to oases, supported by artesian water, and to wadi channels where episodic rainstorms produce transient communities of annuals and herbaceous perennials[4].

Table 1 Radiocarbon dates from the Oyo sediments

Site and depth from surface (cm)	Material analysed	Radiocarbon laboratory number	Age (yr BP)
Hole-6, 170–180	Algal sapropel	SMU-1232	4,920 ± 200
Hole-6, 270–280	Algal sapropel	SMU-1233	5,880 ± 80
Hole-6, 310–320	Algal sapropel	SMU-1231	6,100 ± 80
Hole-6, 460–470	Algal sapropel	SMU-1230	8,490 ± 90
Hole-6, 460–470	Charcoal	AA-253	8,030 ± 280
Auger-1, 460	Charcoal	A-2826	8,900 ± 120
Auger-1, 180	Algal sapropel	A-2820	4,590 ± 140
Well-2, 200	Charcoal	A-2951	8,720 ± 170

The auger-1 site samples were collected from the basin sediments 150 m north-west of the hole-6 pit; the well-2 charcoal sample was recovered from the base of the Zone I sediments exposed in a hand-dug well 260 m west of the hole-6 pit, at a depth of 200 cm from the surface, near the margin of the basin. The analyses were conducted at the Radiocarbon Laboratory of Southern Methodist University, Texas (SMU) and at the Arizona National Science Foundation Regional Facility for Accelerator Mass Spectrometry, Tucson, Arizona (A).

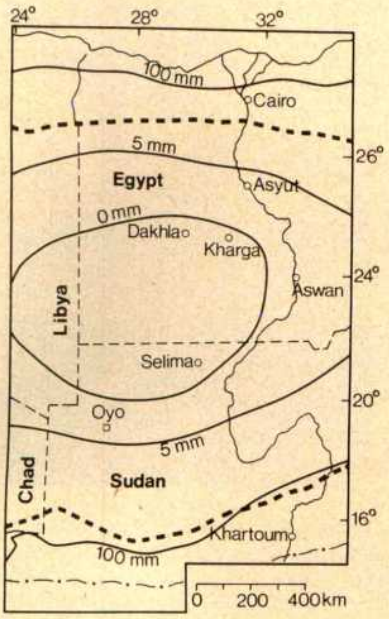


Fig. 1 Map of the Eastern Sahara region showing the location of the Oyo site, the 0, 5 and 100-mm mean annual precipitation limits (after Dubief[2]), the northern and southern limits of absolute desert (- - -) and the northern limit of the savanna (-·-·-·-) (both after White[4]).

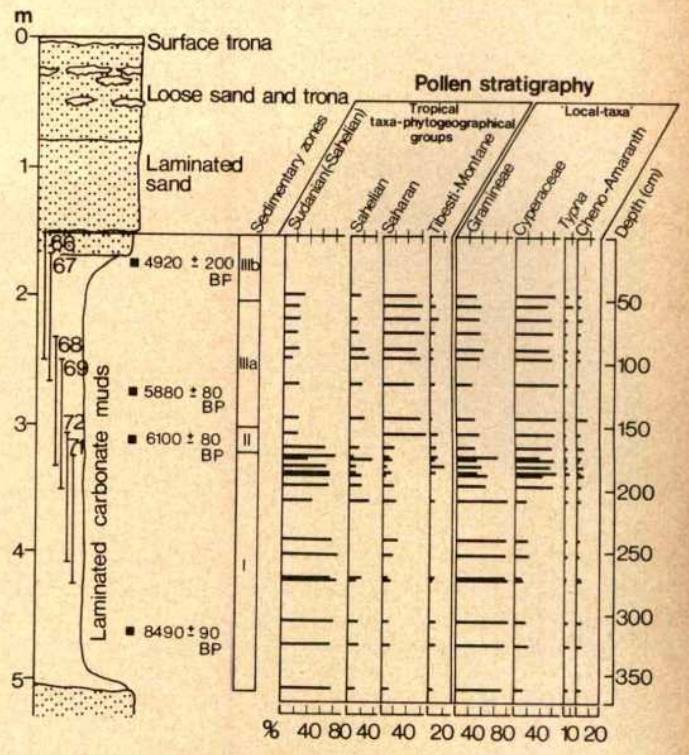


Fig. 2 A summary diagram showing the sediment stratigraphy, positions of the hole-6 1-m tray samples (numbered), positions and values of the radiocarbon age estimates and the pollen stratigraphy of the main groups. The phytogeographical pollen groups are expressed as percentages of the tropical taxa and the sum of the local taxa is the pollen total for the 'local' percentages.

The sample site is in a small basin, ~1.0×0.5 km in size, in a 2-km wide depression open to the south-west and bounded by sandstone escarpments. The sediments were discovered in 1980 and sampled in 1982 and 1983, by hand augering and by digging a 5-m deep pit (hole-6 pit) in the centre of the basin[1]. Undisturbed overlapping 1-m lengths of sediment were recovered from the pit face in aluminium trays each 5 cm in width and depth. The lowest levels were collected by discontinuous bulk sampling because groundwater made tray sampling impossible. Four levels in hole-6 pit and three from nearby auger holes were dated by radiocarbon analysis (Fig. 2, Table 1). The radiocarbon dates of algal sapropel can be considered to be maximum values because of the possibility of error due to older carbonates. The charcoal from the 460–470-cm level in hole-6, dated by the tandem accelerator mass spectrometer (TAMS), indicates a potential discrepancy, but the large standard deviation precludes any firm conclusions about the magnitude of the carbonate error.

Sedimentological analysis suggested that the sequence can be divided into three major zones[5]. Zone I consists predominantly of finely laminated (0.1–1.0-mm) carbonate muds interbedded with sequences of pure white and grey laminae, dark organic bands and bioturbated horizons. The finely laminated muds are composed of green/brown and cream microcrystalline dolomite with trace amounts of clay minerals. Annual deposition of these couplets is suggested by comparing sedimentation rates estimated from ^{14}C dates (Fig. 2; 1 cm every 11–15 yr) with the counted average of 13 couplets cm^{-1}. Units of pure white and grey laminations consist predominantly of calcite with minor amounts of quartz and dolomite and are always associated with dark organic-rich bands composed of algal remains (Fig. 3).

Zone I sediments indicate carbonate deposition in a stratified lake with stagnant bottom waters[6–8]. The predominance of dolomite with an olive-green colour and absence of burrowing organisms through most of this zone suggests reducing conditions, characteristic of arid perennial lakes receiving a restricted seasonal supply of fresh water[8]. The association of white and grey laminations, organic bands and bioturbated horizons (Fig. 3) suggests that episodic increased inflow of calcium-bearing freshwater into the basin and/or temperature fluctuations are responsible for triggering calcite precipitation, algal blooms and weakening of the halocline.

Zone II sediments consist of laminated carbonate muds similar to the green/brown and cream laminations of zone I but with irregularities in thickness, abundant soft sediment deformation structures and evidence of downslope resedimentation and slumping of sediment blocks. Such increased sediment instability may have been caused by lowered lake levels.

Zone III consists of a coarsening upwards sequence of highly deformed muds and sands. The deformed and crudely bedded dolomitic muds of zone IIIa contain significant amounts of windblown sand, which may indicate shallowing of the lake body. Final desiccation is indicated by the crudely bedded and bleached aeolian sands of zone IIIb (Fig. 2).

So far, 55 pollen taxa have been recorded (by J.C.R.) in the sediments, belonging to the following phytogeographical groupings[9–12]: (1) Sudano-Sahelian taxa, found today in the tropical savannas of north-central Sudan and adjacent territories of Africa; (2) Sahelo-Saharan taxa with modern distribution in the thorn scrub and herbaceous desert belt of North Africa; (3) Saharan taxa, today confined to the Sahara and adjacent Arabian deserts. (These tropical taxa ((1)–(3) above) are listed in Table 2.) (4) Tibesti-Montane elements, so designated because the nearest pollen source is the Tibesti Massif, represented here by *Artemisia*, *Ephedra* (two types) and *Erica arborea*; (5) Mediterranean taxa, represented by rare occurrences in a few levels of *Betula*, *Picea*, *Pinus* and *Quercus*; and (6) a group of taxa of uncertain geographical affinity (Gramineae, Cyperaceae, *Typha*, Compositae–Asterae, Compositae–Fenestratae, Chenopodiaceae–Amaranthaceae, Leguminosae and *Myriophyllum*). Many of the tropical taxa are plants with low pollen productivity (for example *Piliostigma*, *Grewia*) and, in addition, several produce large (>60-μm) pollen grains with low dispersal capacity (*Acacia*, *Abutilon*, *Delonix*, *Hibiscus*, *Commicarpus*, *Grewia tenax* and *Pavonia*); thus, although they occur in low concentrations (100–500 grains ml^{-1}), their presence, in a state of excellent preservation, suggests that the source plants were close to, or in, the sedimentary basin.

Table 2 Tropical pollen taxa recovered from the sediments, grouped according to their phytogeographical affinities

Sudanian (-Sahelian)	Sahelian (Sudanian)	Saharan
Acacia cf. *sieberana*	*Abutilon*	*Aerva*
Borreria radiata	*Acacia* cf. *nilotica*	*Cleome*
Boscia	*Acacia* cf. *senegal*	*Maerua*
Cadaba	*Acacia* cf. *seyal*	*Salvadora*
Celtis integrifolia	*Acacia* cf. *raddiana*	*Tamarix*
Chlorophytum	*Atractylis*	
Commicarpus	*Balanites*	
Commiphora	*Blepharis*	
Delonix regia	*Borreria* cf. *chaetocephala*	
Grewia cf. *tenax*	*Capparis*	
Hibiscus (2 taxa)	*Cassia*	
Lannea	*Chroxophora*	
Pavonia	Combretaceae	
Piliostigma cf. *thonningii*	*Corchorus*	
Piliostigma cf. *reticulatum*	*Ottelia*	
Ruellia	*Phyllanthus maderspatensis*	
Zizyphus	*Polygala* cf. *erioptera*	

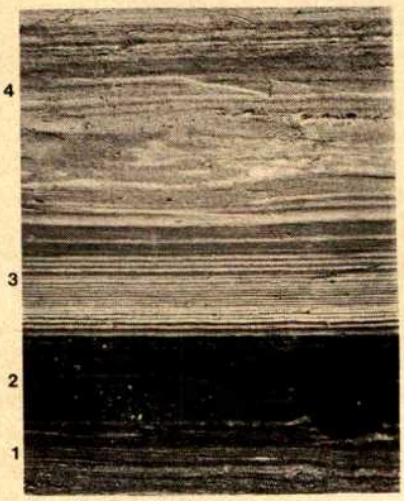


Fig. 3 Segment of Zone I (188–193 cm) sediments, showing: (1) finely laminated green/cream dolomite-rich muds; (2) finely laminated dark organic unit; (3) pure white/grey laminae, thinning upwards; (4) bioturbated laminated muds. Sample hole is 1 cm in diameter.

Gramineae and Cyperaceae, the dominant taxa in all samples, have pollen concentrations between 10,000 and 60,000 grains ml^{-1}, indicating both a regional and local component.

The pollen stratigraphy shows a close correlation with the sediment zones (Fig. 2). The lower levels, 360–170 cm, exhibit a predominance, among the tropical taxa, of Sudano-Sahelian elements of which *Grewia tenax* and *Piliostigma*, both tree taxa characteristic of the modern deciduous savanna zone, are notable. Zones II and III of the sediment column are characterized by an abrupt increase in Sahelian and Saharan elements, typical of modern Sahelian savannas and thorn scrub vegetation. A significant change in the proportions of Gramineae and Cyperaceae occurs at approximately the Zone I/II boundary (Fig. 2). This change is difficult to interpret, but the relative increase in Cyperaceae could have been caused by a lowered lake level producing a larger marginal marshy zone; alternatively, part of the increase may have been the result of an expansion of such important dune taxa as *Cyperus conglomeratus*.

The sediment, pollen and radiocarbon data from this site indicate (1) that between 8,500 and 6,100 yr BP the Oyo depression supported a relatively deep stratified lake with stagnant bottom water and annual inputs of fresh water, surrounded by a continuous deciduous savanna vegetation, similar to that found today ~500 km to the south; (2) that at about 6,000 yr BP a reduction in precipitation and/or an increase in evaporation initiated a shallowing of the lake; and (3) that between 6,000 and 4,500 yr BP the lake became still shallower and the tropical Sudano-Sahelian savannas were replaced by *Acacia*-thorn savanna and scrub-grassland. Finally, at 4,500 yr BP the lake basin dried out and was covered subsequently by aeolian sediments, whereas the vegetation, in the form of desert scrub-grasslands, disappeared from all habitats except at oases and wadis.

The Holocene lake at Oyo was probably groundwater supported, as at Merga Lake, 40 km to the south-east, today[13]. Recent isotope investigations of shallow groundwater in the region support a model of direct recharge[14–16] through the oases depressions, as opposed to subsurface flow over great distances, such as from the Enedi highlands in the case of Oyo.

The palaeoclimates suggested by the above conclusions are: for Zone I, a humid tropical climate with annual monsoonal rainfall of at least 400 mm; for Zones II and III, a progressive increase in aridity with annual precipitation declining from 300 mm at 6,000 yr BP to <100 mm at 4,500 yr BP. These reconstructions corroborate previous conclusions based on evidence from laminated lake sediments[17–20], East Mediterranean sapropels[21], geomorphology and archaeology[22,23] and Nile River flood history[24]. Our results provide the first conclusive demonstration of vegetation and climate change in the early to mid-Holocene of the eastern Sahara. They agree with tentative conclusions of Maley[25–27] for the distant Lake Chad record, with the general Holocene lake level trends for north-east Africa[28,29] and with changes in the position of the African monsoon predicted on the basis of Milankovitch orbital forcing factors[30,31]. Detailed sediment, radiocarbon and pollen analyses of the Oyo deposits will be reported elsewhere.

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1. Haynes, C. V. *Natn. Geogr. Soc. Res. Rep.* **16** (in the press).
2. Dubief, J. *Mém. Inst. Rech. Sah. Univ. Alger* **2**, 1–275 (1963).
3. Haynes, C. V. *Natn. Geogr. Soc. Res. Rep.* **15**, 257–293 (1983).
4. White, F. *The Vegetation of Africa* (UNESCO, Paris, 1983).
5. Eyles, C. H., Haynes, C. V. & Ritchie, J. C. *Geol. Soc. Am. Abstr.* **16**, 505 (1984).
6. Wasson, R. J., Smith, G. I. & Agrawal, D. P. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **46**, 345–372 (1984).
7. Eugster, H. P. & Hardie, L. A. in *Lakes: Chemistry, Geology Physics* (ed. Lerman, A.) 237–293 (Springer, New York, 1978).
8. Smith, G. I. *US Geol. Surv. Prof. Pap.* **1043**, 130 (1979).
9. Wickens, G. E. *The Flora of Jebel Marra* (HM Stationery Office, London, 1976).
10. Lebrun, J.-P. *Eléments pour un Atlas des Plantes Vasculaires de l'Afrique sèche* Vol. 1 (Inst. d'élevage et de médecine vétérinaire des pays tropicaux, Maisons Alfort, 1977).
11. Lebrun, J.-P. *Eléments pour un Atlas des Plantes Vasculaires de l'Afrique sèche* Vol. 2 (Inst. d'élevage et de médecine vétérinaire des pays tropicaux, Maisons Alfort, 1979).
12. Lebrun, J.-P. *Catalogue des Plantes Vasculaires du Niger* (Inst. d'élevage et de médecine vétérinaire des pays tropicaux, Maisons Alfort, 1976).
13. Haynes, C. V., Mehringer, P. J. & Zaghloul, S. A. *Geogr. J.* **145**, 437–445 (1979).
14. Haynes, C. V. & Haas, H. *Radiocarbon* **22**, 705–717 (1980).
15. Muller, A. B. & Haynes, C. V. in *Isotope Hydrology in Water Resources Development* (IAEA, Vienna, 1984).
16. Thoreweihe, U., Schneider, M. & Sonntag, C. *Berliner Geowissenschaftliche Abhandlungen* **50**, 209–216 (1984).
17. Haynes, C. V. *Geol. Soc. Am. Abstr.* **14**, 511 (1982).
18. Mehringer, P. J. Jr *Geol. Soc. Am. Abstr.* **14**, 512 (1982).
19. Baumhauer, R. & Schulz, E. *Palaeoecol. Africa* **16**, 283–289 (1984).
20. Gabriel, B. & Kropelin, S. *Palaeoecol. Africa* **16**, 295–299 (1984).
21. Rossignol-Strick, M. *Nature* **303**, 46–49 (1983).
22. Haynes, C. V. *Science* **217**, 629–633 (1982).

23. Haynes, C. V. in *Prehistory of the Eastern Sahara* (ed. Wendorf, F. & Schild, R.) 353–371 (Academic, New York, 1980).
24. Adamson, D. A., Gasse, F., Street, F. A. & Williams, M. A. J. *Nature* **287**, 50–55 (1980).
25. Maley, J. *Etudes palynologiques dans le bassin du Tchad et paléoclimatologie de l'Afrique nord-tropicale de 30 000 ans à l'époque actuelle* (Travaux et Documents de L'ORSTOM 129, Paris 1981).
26. Maley, J. *Nature* **269**, 573–577 (1977).
27. Maley, J. *Bothalia* **14**, 377–389 (1983).
28. Street, F. A. & Grove, A. T. *Nature* **261**, 385–390 (1976).
29. Street, F. A. & Roberts, N. in *Variations in the Global Water Budget* (ed. Street, F. A. *et al.*) 331–345 (Reidel, Dordrecht, 1983).
30. Kutzbach, J. E. *Quat. Res.* **14**, 210–223 (1980).
31. Kutzbach, J. E. & Otto-Bliesner, B. L. *J. atmos. Sci.* **39**, 1177–1188 (1982).

A terrestrial fauna from the Scottish Lower Carboniferous

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Despite several important discoveries, extending over more than 120 years, our knowledge of early land vertebrates is still sparse. The earliest tetrapod remains are known from the Upper Devonian of East Greenland[1–3] and Australia[4–6], but the tetrapod fossil record does not become plentiful until Coal Measure times, in the Upper Carboniferous, some 50 Myr later. Finds in the Lower Carboniferous are very few indeed. Apart from two localities in West Virginia, USA[7,8], and one in Nova Scotia, Canada[9], all other Lower Carboniferous tetrapod sites are from the Viséan of Fife and the Lothian Region, Scotland[10,11]. We report here the discovery of an assemblage of terrestrial animals from a new Lower Carboniferous locality in the Lothian Region. Specimens were collected from the East Kirkton Limestone in the Brigantian stage of the Scottish Viséan, and include the first articulated amphibian skeleton to be found in the Lower Carboniferous of Europe in the twentieth century. This find is the earliest well-preserved amphibian skeleton ever discovered. The associated fauna is remarkable for the presence of myriapods, scorpions, the earliest known harvestman and several other types of amphibian. The presence of such forms, together with the striking absence of fishes, suggests that the amphibians form an integral part of a terrestrial fauna; terrestrial amphibians are otherwise unknown before the Upper Carboniferous Coal Measures.

The earliest Lower Carboniferous tetrapod sites are in the Oil Shale Group (Fig. 1), and the fauna consists of the aïstopod *Lethiscus*[12], the colosteid temnospondyl *Pholidogaster*[13] and a form of unknown affinities, *Doragnathus*[14]. There is also a series of specimens, including three incomplete skeletons, which are attributable to the Adelogyrinidae[15]. The principal amphibian find reported here is not attributable to any of these taxa. The site from which it comes is a new one in the Lothian Region. The specimen was found in the East Kirkton Limstone in sediments originating from the East Kirkton Quarry, near Bathgate, West Lothian. The limestone is very localized and separated by the Riccarton Hills Lavas from the overlying Hurlet Limestone, which marks the boundary between the Upper Oil Shale Group and the Lower Limestone Group (Fig. 1)[16]. The underlying Bankhead Tuffs separate it from the Raeburn Shell Bed, which in West Lothian probably forms the base of the Brigantian[17].

In striking contrast to other Scottish Mississippian tetrapod localities, there is a complete absence of fish remains at this horizon. The matrix in which the amphibians were found is a peculiar laminated cherty limestone[18], and initial associated discoveries suggest a unique environment of deposition. Most notable among these is what appears to be the earliest known fossil harvestman (Arachnida: Opiliones)[19] (Fig. 2). A number of myriapods and scorpions have also been found. Together with these small arthropods are several incomplete specimens

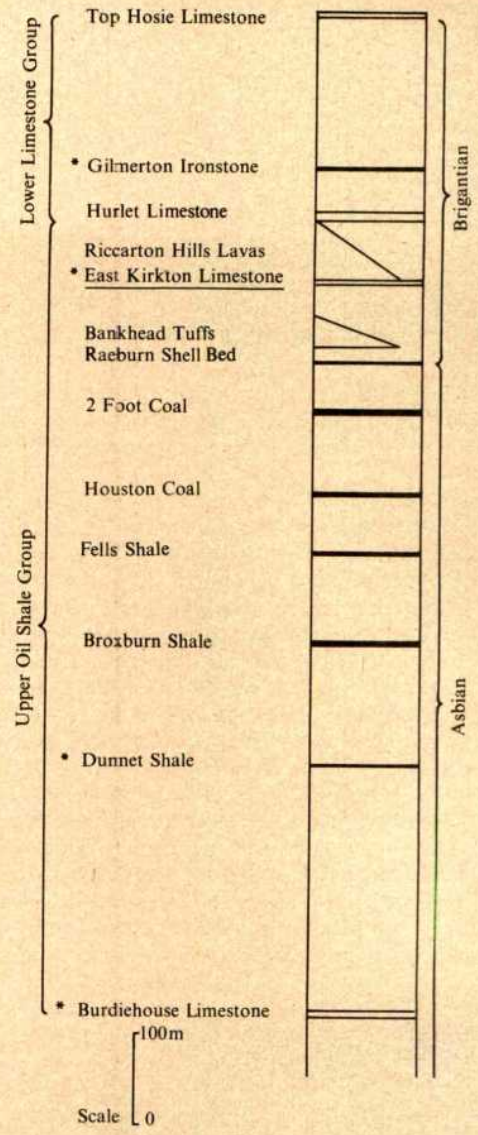


Fig. 1 Generalized section of the Upper Viséan of West Lothian, Scotland (after Geological Survey of Great Britain (Scotland), Sheet 32W). *Amphibian horizons.



Fig. 2 Fossil harvestman (Opiliones) from the East Kirkton Limestone (SPW: G480). Natural size.

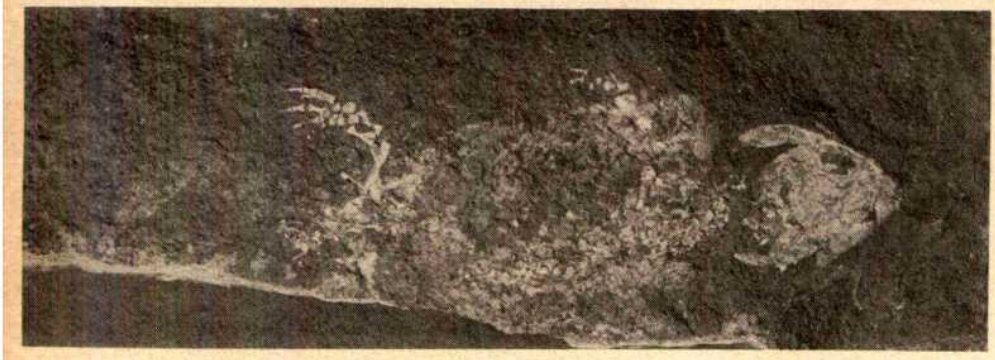


Fig. 3 The earliest complete fossil amphibian skeleton (SPW: 2042A). Dorsal view (×~0.4).

of gigantic eurypterids. *Hibbertopterus scouleri* has already been described from the East Kirkton Limestone[20], but the new material cannot be referred to any Scottish Lower Carboniferous species.

The principal find (field no. SPW: 2042 A/B) consists of a tetrapod skeleton of some 40 cm overall length. It is preserved in part and counterpart on two blocks of limestone. The principal block (Fig. 3) shows the skeleton as it was when originally collected. The skull, vertebral column, girdles and limbs are all in natural articulation and the specimen is completed by the tail, which is defined by its investing scales. The specimen is remarkable for the intact preservation of the hands and feet, with ossified carpals and tarsals, and should greatly augment our understanding of the early evolution of the tetrapod limb.

The skull is similar to those of edopoid and dissorophoid temnospondyls from the Upper Carboniferous and Lower Permian[21,22]. The orbits and interpterygoid vacuities are relatively large, the otic notches are distinct and there is no visible trace of a lateral line canal system. However, the sutures between the bones of the skull roof and palate are difficult to trace and a detailed study will be necessary before the relationships of this new species can be established. The vertebrae appear to be of the primitive temnospondyl type, with separate intercentra and pleurocentra surrounding a persistent notochord. The general morphology of the appendicular skeleton is also similar to that of temnospondyls, with a four-digit manus and five-digit pes, but the structure of the carpus and tarsus, as well as the phalangeal formulae of the hands and feet, remain to be determined. Six other specimens from the East Kirkton Limestone are provisionally attributed to this species. They include a skull of similar size to that of the complete skeleton (Fig. 4), a pelvis with paired hind limbs, and two incomplete skeletons.

A second temnospondyl species is represented in the fauna by an incomplete and poorly preserved skeleton. It differs from the complete skeleton illustrated in Fig. 3 in being much shorter-bodied and with limbs of different proportions. Anthracosaur amphibians have also been found. Caudal vertebrae, similar to those of *Proterogyrinus*[23], have been collected, together with two incomplete humeri which resemble closely those of *Eoherpeton*[24].

There is no sign in the complete amphibian skeleton of an aquatic mode of life; lateral-line canals seem to be absent and the skeleton, particularly that of the limbs, is remarkably heavily ossified. This is also true of the other amphibian remains, and, in the light of the associated arthropods, it seems probable that they represent the only known pre-Coal Measure terrestrial vertebrate fauna. Complete preparation and description of this new material is under way, but will take some time. Meanwhile, we anticipate that further exploration of the East Kirkton Limestone will yield more evidence of the early Carboniferous land fauna. It is hoped that this will result in the discovery of the forerunners of the many amphibian groups which appear suddenly in the Upper Carboniferous. We do not exclude the possibility of the discovery of the earliest reptiles.

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a



b



Fig. 4 Fossil amphibian skull (SPW: 2049). *a*, Skull roof, ventral view; *b*, palate, dorsal view. Natural size.

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1. Säve-Söderbergh, G. *Meddr Grønland* **94**, No. 7, 1–107 (1932).
2. Jarvik, E. *Ark. Zool.* **41A**, No. 13, 1–8 (1948).
3. Jarvik, E. *Meddr Grønland* **149**, No. 6, 1–20 (1950).
4. Warren, J. W. & Wakefield, N. A. *Nature* **238**, 469–470 (1972).
5. Campbell, K. W. S. & Bell, M. W. *Alcheringa* **1**, 369–381 (1977).
6. Warren, A. A. *The Fossil Vertebrate Record of Australasia* (eds Rich, P. V. & Thompson, E. M.) 145–157 (Monash University Press, 1982).
7. Romer, A. S. *Proc. Am. phil. Soc.* **100**, 157–167 (1956).
8. Smithson, T. R. *Zool. J. Linn. Soc.* **76**, 29–90 (1982).
9. Carroll, R. L. *et al. Field Excursion A59: Guidebook* (24th int. Geol. Congr., Montreal, 1972).
10. Paton, R. L. *R. Scott. Mus. Inform. Ser. Geol.* **5**, 1–38 (1975).
11. Andrews, S. M., Browne, M. A. E., Panchen, A. L. & Wood, S. P. *Nature* **265**, 529–532 (1977).
12. Wellstead, C. F. *Palaeontology* **25**, 193–208 (1982).
13. Panchen, A. L. *Phil. Trans. R. Soc.* **B259**, 581–640 (1975).
14. Smithson, T. R. *Palaeontology* **23**, 915–923 (1980).
15. Brough, M. C. & Brough, J. *Phil. Trans. R. Soc.* **B252**, 107–165 (1967).
16. Mitchell, G. H. & Mykura, W. *The Geology of the Neighbourhood of Edinburgh* (HMSO, Edinburgh, 1962).
17. George, T. N. *et al. A Correlation of Dinantian Rocks in the British Isles* (Geological Society, London, 1976).
18. Muir, R. O. & Walton, E. K. *Trans. geol. Soc. Glasg.* **22**, 157–168 (1957).
19. Petrunkevitch, A. *Treatise on Invertebrate Paleontology* Pt P, Vol. 2 (ed. Moore, R. C.) 44–162 (Geological Society of America, Kansas, 1955).
20. Waterston, C. D. *Trans. R. Soc. Edinb.* **63**, 265–288 (1957).
21. Carroll, R. L. *J. Paleont.* **41**, 111–142 (1967).
22. Milner, A. R. *Palaeontology* **25**, 635–664 (1982).
23. Holmes, R. *Phil. Trans. R. Soc.* **B306**, 431–524 (1984).
24. Smithson, T. R. *Zool. J. Linn. Soc.* (in the press).

Multiple innervation of tonic endplates revealed by activity-dependent uptake of fluorescent probes

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During development of the vertebrate nervous system, there is a widespread reduction in the number of axons innervating target cells[1]. This phenomenon, often called synapse elimination, has been particularly well studied at the neuromuscular junction of developing twitch muscle fibres[2,3]: following a period of polyneuronal innervation, axonal branches are retracted, usually leaving each twitch fibre endplate innervated by only one axon. Here we describe a new technique for the study of synapse elimination—activity-mediated uptake of fluorescent probes. These probes selectively and supravitally label all the terminals of individual axons. The technique is used here in adult and embryonic snakes to study the innervation pattern of a thin muscle containing two fibre types: twitch fibres, which are fast-contracting and have propagated action potentials, and tonic fibres, which are slow-contracting and lack action potentials. We find that twitch muscle fibres, as expected, eliminate all polyneuronal innervation during development; in contrast, tonic fibre endplates remain polyneuronally innervated into adulthood. The persistence of multiple innervation at tonic endplates may be related to the lack of action potential activity in tonic muscle fibres.

The labelling technique takes advantage of endocytosis at the synaptic terminals of stimulated axons[4] to accumulate fluorescent molecules at synapses. Our method allows living terminals to be viewed with light microscopy, but, unlike other supravital techniques[5,6], the synapses of different axons in the same preparation can be stained selectively with different-coloured probes.

We have at present three fluorescent probes that are internalized selectively by active nerve terminals; they are sulphonic acid derivatives of fluorescent molecules of low relative molecular mass (rhodamine, fluorescein and 8-hydroxypyrene; see Fig. 1 legend). Each probe emits at a different peak wavelength (red, green and blue, respectively). Brief stimulation (5–10 min) of a muscle nerve in a bath containing one of the probes labels all the terminals of the stimulated motor axons. Sequential stimulation of individual motor axons in the presence of different-coloured probes allows the relationship among terminals of different axons to be visualized.

The innervation of twitch and tonic muscle fibre endplates was examined in the transversus abdominis muscle of the garter snake; this muscle comprises ~200 segmental components, each of which extends from a rib to the ventral midline of the animal[7]. Each segmental component is supplied by a nerve containing 4–5 twitch and 3–4 tonic motor axons. The muscle is only one fibre thick, thus all the nerve–muscle contacts are visible in living preparations. A useful feature of this muscle is that the segmental components form an uninterrupted sheet, so that adjacent segmental nerves occasionally innervate the same band of endplates. By stimulating one segmental nerve in the presence of one fluorescent probe and then stimulating the adjacent nerve in the presence of a different-coloured probe, we could label separately all the terminals of two populations of axons in the same endplate band in either adult or embryonic snakes.

Initially the probes were used to study the pattern of innervation at the border region between adjacent segmental muscles. The results (Fig. 1*a*) showed that twitch fibre endplates innervated by one segmental nerve (red) are interspersed with endplates innervated by the adjacent segmental nerve (green). None of the individual twitch fibre endplates showed a mixture of red and green boutons, which would have indicated innervation by axons from both nerves.

In contrast, tonic muscle fibre endplates in adult snakes showed a very different pattern of labelling: when one of the segmental nerves was stimulated in the presence of the red probe and the adjacent nerve in the presence of the green probe, many of the tonic fibre endplates near the segmental border contained a mixture of both red and green synaptic boutons (Fig. 1*b*). Such 'mixed' endplates were found in each of 15 preparations in which there was a continuous endplate band crossing segmental boundaries. This observation indicates that, in maturity, tonic fibre endplates near the segmental boundaries are frequently innervated by axons from both segments.

To determine whether multiple innervation is typical of tonic fibre endplates throughout the muscle rather than just at the borders, we labelled a single tonic motor unit with the red probe, and all the other motor units within that segment with the blue probe (Fig. 1*c*). In this way we could examine every endplate site contacted by a single tonic motor axon. Each of two tonic units (from two snakes) studied in this way contributed 25–30% of synaptic boutons on the tonic muscle fibres in the muscle (1,477/5,539 and 1,305/4,571), yet each innervated 44% of the total number of tonic fibre endplates (98/223 and 102/234), suggesting that many of the endplates are multiply innervated. In fact, in both motor units, most of the endplates having red boutons, and thus innervated by the separately stimulated motor unit (85% and 54%), were also innervated by other axons as demonstrated by the presence of blue boutons at the same endplate (Fig. 1*c*). In contrast, three experiments in which a twitch motor unit was selectively labelled in the same manner as the tonic units (see above) revealed uniformly single innervation of twitch fibres: none of the twitch fibre endplates having red boutons, and thus within the separately stimulated twitch motor unit, also had blue boutons.

To estimate the number of axons that converge at a tonic fibre endplate, the numbers of red and blue boutons were counted at each tonic endplate site. The fraction of the total number of boutons contributed by the separately stimulated motor unit [red/(red + blue)] indicates the percentage of that endplate supplied by one axon and thus provides an approximation of the number of innervating axons. At multiply innervated endplate sites (those with both red and blue boutons), the identified motor axon formed, on average, less than half of the boutons (46% and 23%), arguing that many tonic fibre endplates are doubly innervated and occasional endplates are innervated by more than two axons.

The anatomical evidence for single innervation of twitch fibres and multiple innervation of tonic fibres was confirmed electrophysiologically. Intracellular recordings at the endplate sites of 50 twitch fibres (paralysed with curare or formamide[8]) always showed a single step in the postsynaptic response to a gradually increasing strength of stimulus to the muscle's nerve; a single step indicates recruitment of only one innervating axon (Fig. 2*a*). Physiological confirmation of multiply innervated tonic fibre endplates is more complicated as each tonic muscle fibre has several endplates[9,10]. Thus, both the presence of multiple steps in the postsynaptic response to gradually increasing nerve stimulation and the risetime of these steps must be considered to obtain unambiguous evidence for multiple inputs at a single endplate site. Intracellular recordings from tonic muscle fibres immediately adjacent to one endplate site (located with Nomarski optics) showed the presence of such multiple innervation (Fig. 2*b–d*). Of 57 tonic endplates studied electrophysiologically, 49% seemed to be singly innervated (see Fig. 2*b*), 44% doubly (see Fig. 2*c*) and 7% triply innervated (Fig. 2*d*), all consistent with the degree of multiple innervation assessed anatomically.

Studies of developing snake muscles suggest that the differing innervation patterns of twitch and tonic fibre endplates arise during development because of more vigorous synapse elimination from twitch than from tonic muscle fibres. The transversus abdominis muscle was dissected from late embryonic snakes (stages 35–36)[11]. The activity-selective fluorescent probes worked well in embryos, although endplates were smaller than

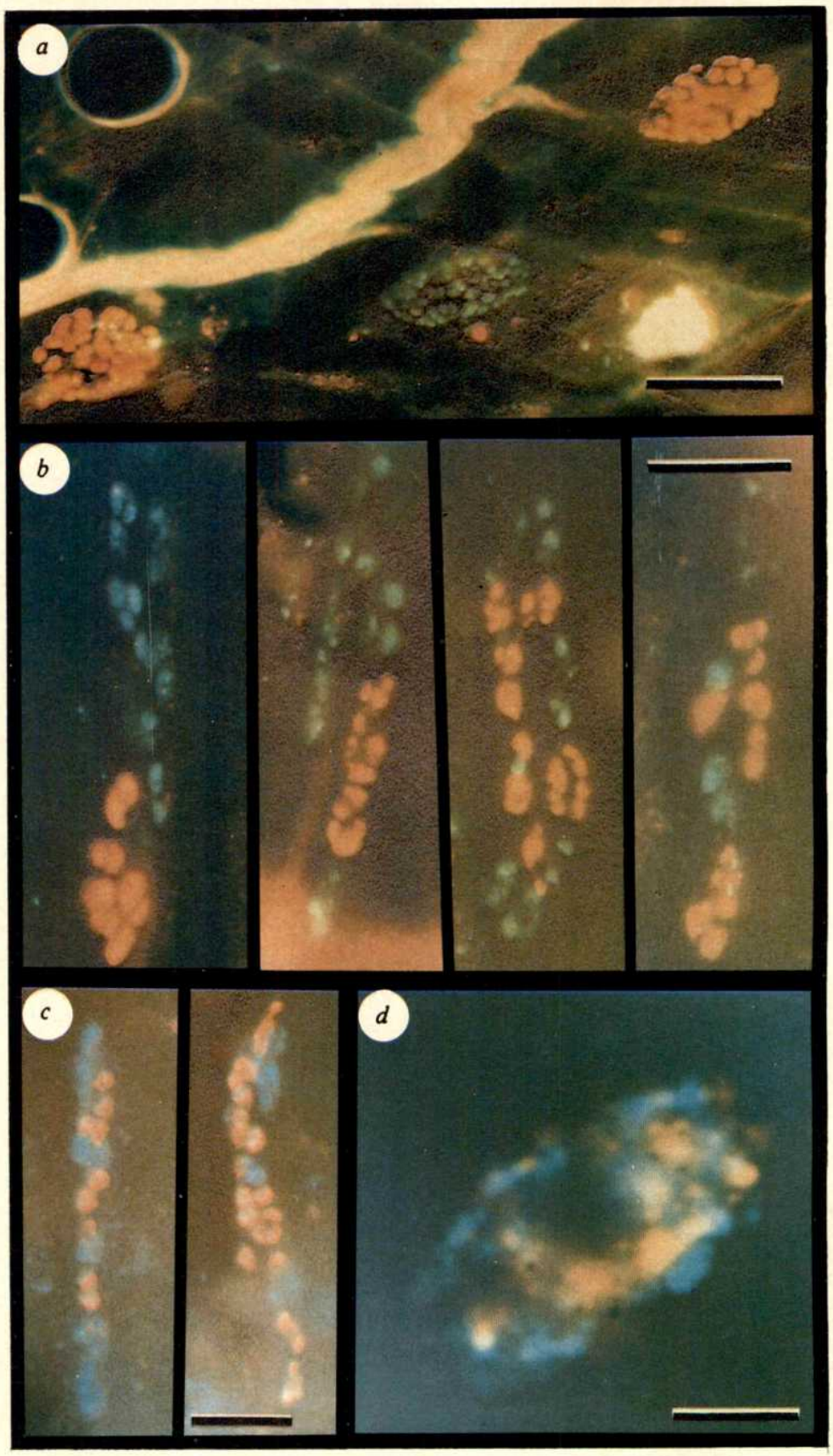


Fig. 1 Photomicrographs of activity-mediated uptake of fluorescent probes by motor axon terminals in the adult and embryonic transversus abdominis muscle of the garter snake *Thamnophis sirtalis*. ***a***, At the segmental boundary, adult twitch muscle fibres innervated by adjacent segmental nerves are interspersed. None of the twitch terminals studied was innervated by both segmental nerves. The figure shows two red-labelled nerve terminals supplied by the more rostral segmental nerve (left); between them is a green terminal from the more caudal segmental nerve (right). The rostral segmental nerve was stimulated for 5 min (75 Hz, 3 s on/2 s off) in a bath containing Ringer's solution and sulphorhodamine 101 (0.1 mg ml^{-1}; Molecular Probes Inc., Junction City, Oregon). The preparation was washed in Ringer's for 30 min, placed in Ringer's containing 0.1 mg ml^{-1} fluorescein 5,6-sulphonic acid (Molecular Probes Inc.), and the adjacent (caudal) nerve stimulated in the same manner. The preparation was washed for 30 min, fixed in 4% paraformaldehyde for 30 min and mounted on a slide in *p*-phenylenediamine phosphate-buffered saline/glycerin solution[23] to prevent fading. Living material may also be used but exciting light levels must be kept low to prevent bleaching and damage. Calibration bar, 35 μm. ***b***, Individual tonic endplates at the border of adjacent segmental muscles are frequently innervated by axons from both segmental nerves. With the same stimulus paradigm as in *a*, most tonic endplates in the border region showed a mixture of green and red boutons. Farther from the segmental border, tonic endplates containing both types of boutons declined, becoming all red in the rostral segment and all green in the caudal segment. Calibration bar, 10 μm. ***c***, The synaptic contribution of one motor axon to multiply innervated tonic endplates. To assess the proportion of synapses contributed by one axon in a multiply innervated endplate, the boutons of all the motor axons were labelled with the blue fluorescent probe 8-hydroxypyrene trisulphonic acid (Eastman Organic Chemicals, Rochester, New York) by stimulating the segmental nerve at supramaximal strength (same procedure as above); boutons of a single tonic motor axon were subsequently filled with the red probe (and, concomitantly, partially depleted of the blue probe) by selectively restimulating it alone in the presence of sulphorhodamine 101. A single tonic motor unit was activated by locating a tonic axon near an endplate (with Nomarski microscopy) and stimulating it with an extracellular electrode. This stimulation technique retrogradely activates the entire motor unit[24]; extracellular recording from the proximal nerve showed a single action potential, thus only one motor axon was active. The figure shows two of the 102 endplates innervated by the stimulated tonic axon (the boutons of the separately stimulated axon are red; the rest are blue). Calibration bar, 10 μm. ***d***, Multiple innervation of a twitch endplate in an embryonic snake muscle. Using the same stimulus procedure as in *a* and *b*, the motor axons from adjacent segments were labelled with the red and blue probes. Although all endplates were smaller than in adults, twitch endplates were still recognizable by virtue of their round shape and larger size than tonic endplates. Unlike adult preparations, embryonic twitch endplates at the segmental border received input from both segments, indicating innervation by more than one axon. Calibration bar, 5 μm. *a–c*, Photomicrograph double exposures using two sets of barrier and excitation filters. *d*, Photograph of a digitally stored video image.

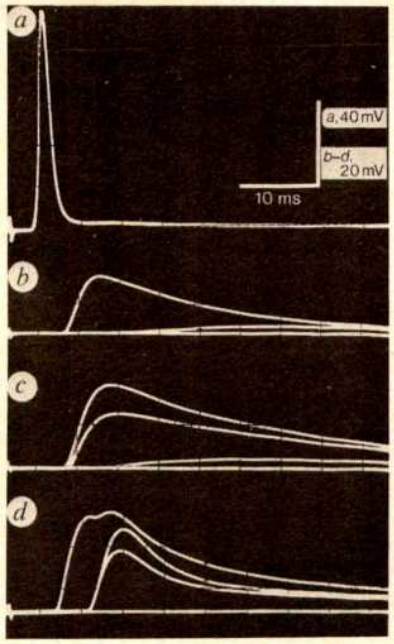


Fig. 2 Intracellular recordings from adult twitch and tonic muscle fibres in the transversus abdominis muscle of garter snakes, treated with formamide to induce muscle paralysis without reducing the amplitude of synaptic potentials[8]. *a*, Twitch fibres were identified by their large endplates. Recordings near the endplate always showed a single, large regenerative response in the postsynaptic potential. The unitary postsynaptic response is evidence of innervation by a single motor axon. Similar results were obtained with curare added to the bath in sufficient concentration to make synaptic potentials subthreshold. *b–d*, In tonic fibres, multiple steps in the postsynaptic response to nerve stimulation were often seen. Each panel is an intracellular record from a different tonic muscle fibre; all recordings were made adjacent to a tonic endplate viewed with Nomarski optics. The neighbouring endplate site was singly innervated in *b*, doubly innervated in *c*, and triply innervated in *d*. We interpret the large-amplitude, fast-rising steps as inputs to the nearby endplate, whereas the smaller and slower rising synaptic potentials are presumably inputs to the adjacent endplate sites (at a distance of ~1 mm). No potentials intermediate in risetime and amplitude to the two classes shown here were seen, suggesting that the source of the slow-rising potentials was an adjacent endplate and that more distant synaptic inputs were below the resolution level of our recordings. None of the tonic fibres showed regenerative membrane responses.

in adults and individual synaptic boutons were more difficult to discern. In contrast to the innervation of adult muscles (Fig. 1*a*), both twitch and tonic muscle fibre endplates at the segmental boundary in embryonic muscles showed innervation from both segmental nerves (Fig. 1*d*). Multiple innervation of twitch fibres was found in each of five embryonic muscles studied. However, by 1 week after birth (10 animals) no evidence of multiple innervation of twitch fibre endplates was found using either the fluorescent probes or intracellular recording. This result indicates that synapse elimination at twitch fibre endplates effectively removes all but one input to these muscle fibres perinatally. Tonic muscle fibre endplates, however, showed evidence of polyneuronal innervation throughout development and into adulthood.

It is uncertain why tonic endplates should retain multiple innervation. One possible explanation is suggested by an observation on doubly innervated[12] neuromuscular junctions of some frog twitch fibres—these multiply innervated sites are unusual because the inputs are weak and, when repetitively stimulated, are often unable to depolarize the muscle fibre sufficiently to generate action potentials[12]. Thus, the persistence of multiple innervation at tonic fibre endplates may be related to the fact that tonic muscle fibres generally lack action potential activity[10] regardless of the amplitude of their synaptic inputs (see Fig. 2). Indeed, several experiments suggest that the activity of postsynaptic cells influences synapse elimination[13–20], perhaps by allowing the postsynaptic cell to reward some inputs trophically at the expense of others[21,22]. The ability to view selectively the terminals of living axons undergoing synaptic rearrangement should provide a direct experimental approach to resolving this problem.

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1. Purves, D. & Lichtman, J. W. *Science* **210,** 153–157 (1980).
2. Van Essen, D. C. in *Neuronal Development* (ed. Spitzer, N. C.) 333–376 (Plenum, New York, 1982).
3. Brown, M. C. *et al.* in *Development of the Nervous System* (eds Garrod, D. R. & Feldman, J. D.) 245–262 (Cambridge University Press, 1981).
4. Heuser, J. E. & Reese, T. S. *J. Cell Biol.* **57,** 315–344 (1973).
5. Yoshikami, D. & Okun, L. M. *Nature* **310,** 54–56 (1984).
6. Wilcox, M. & Franceschini, N. *Science* **225,** 851–854 (1984).
7. Wilkinson, R. S. & Lichtman, J. W. *J. Neurosci.* (submitted).
8. Córdoba, F. *et al. J. Physiol., Lond.* **291,** 73–74 p (1979).
9. Bennett, M. R. & Pettigrew, A. G. *J. Physiol., Lond.* **241,** 515–545 (1974).
10. Hess, A. *Physiol. Rev.* **50,** 40–62 (1970).
11. Zehr, D. R. *Copeia* **2,** 322–329 (1962).
12. Angaut-Petit, D. & Mallart, A. *J. Physiol., Lond.* **289,** 203–218 (1979).
13. Benoit, P. & Changeux, J.-P. *Brain Res.* **99,** 354–358 (1975).
14. Riley, D. A. *Brain Res.* **143,** 162–167 (1978).
15. O'Brien, R. A. D. *et al. J. Physiol., Lond.* **282,** 571–582 (1978).
16. Thompson, W. *et al. Neuroscience* **4,** 271–278 (1979).
17. Brown, M. C. *et al. J. Physiol., Lond.* **318,** 355–364 (1981).
18. Duxson, M. J. *J. Neurocytol.* **11,** 395–408 (1982).
19. Thompson, W. *Nature* **302,** 614–616 (1983).
20. Ridge, R. M. A. P. & Betz, W. J. *J. Neurosci.* **4,** 2614–2620 (1984).
21. Changeux, J.-P. & Danchin, A. *Nature* **264,** 705–712 (1976).
22. Purves, D. & Lichtman, J. W. *Principles of Neural Development* (Sinauer, Sunderland, Massachusetts, 1984).
23. Johnson, D. G. *et al. J. immun. Meth.* **43,** 349–350 (1981).
24. Ridge, R. M. A. P. *J. Physiol., Lond.* **217,** 393–418 (1971).

Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes

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The pineal gland has proven to be an excellent model for the study of adrenergic control systems[1]. Noradrenaline, released from sympathetic nerve terminals in the pineal gland, regulates a large nocturnal increase in melatonin synthesis by stimulating the activity of arylalkylamine *N*-acetyltransferase (NAT, EC 2.3.1.87) 30–70-fold[1,2]. An essential step in both the induction and maintenance of high NAT activity is an increase in intracellular cyclic AMP[3,4]. Noradrenaline acts via β-adrenoceptors to increase pineal cyclic AMP by activating adenylate cyclase[5], and the activation of pineal α_1-adrenoceptors[6] potentiates β-adrenergic stimulation not only of NAT[7,8] but of both cyclic AMP and cyclic GMP[9–11]. Here we describe investigations designed to test whether α_1-adrenergic potentiation of β-adrenergic stimulation of pineal cyclic AMP involves protein kinase C. Our results suggest that kinase activation is involved and the data provide the first demonstration of a synergistic interaction between Ca^{2+}-phospholipid-dependent protein kinase (protein kinase C) and neurotransmitter-dependent stimulation of cyclic AMP.

Pinealocytes were prepared from female Sprague–Dawley rats (5 weeks old, 120 g) by trypsinization[12] and incubated (10^5 cells per 0.5 ml, 37 °C, 95% O_2/5% CO_2) in suspension culture in Dulbecco's modified Eagle's medium (DMEM) containing fetal calf serum (10% v/v) for 5–7 h before use. Following the indicated treatments, cells were centrifuged (8,000*g*, 1 min), the

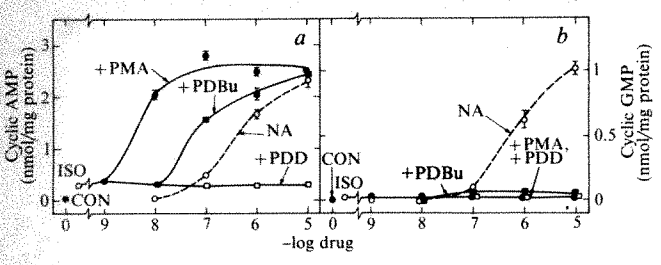


Fig. 1 Potentiation of β-adrenergic cyclic nucleotide responses by phorbol esters (*a*, cyclic AMP; *b*, cyclic GMP). Pinealocytes (10^5 cells per tube) were treated for 15 min with (−)isoprenaline (ISO; 10^{-6} M) either alone or in combination with 4-β-phorbol 12-myristate 13-acetate (PMA), 4-β-phorbol 12,13-dibutyrate (PDBu) or 4-α-phorbol 12,13-didecanoate (PDD). The dose-response curve to (−)noradrenaline (NA) is shown for comparison. Phorbol esters (Sigma) were added as 100 × concentrated solutions in ethanol. All points represent the mean ±s.e.m. of three samples. The lack of error bars indicates that the s.e.m. was less than the area covered by the symbol.

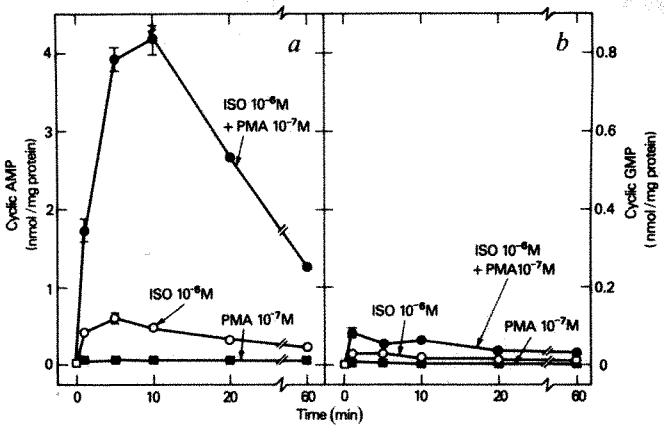


Fig. 2 Time course of PMA potentiation of β-adrenergic cyclic nucleotide response (*a*, cyclic AMP; *b*, cyclic GMP). Pinealocytes (10^5 cells per tube) were treated with PMA (10^{-7} M), (−)isoprenaline (10^{-6} M), or PMA (10^{-7} M) plus (−)isoprenaline (10^{-6} M) for the period indicated. All points represent the mean ±s.e.m. of three samples.

media aspirated and the cell pellet immediately frozen on dry ice. Samples were sonicated in perchloric acid (5%), neutralized, then frozen (−30 °C) until cyclic nucleotide concentrations were determined in duplicate by radioimmunoassay following acetylation[13]. The cross-reactivities of the cyclic AMP and cyclic GMP antisera were 0.07% and 0.53% respectively. Protein was determined by a dye binding assay using bovine serum albumin as the standard[14].

Treatment with isoprenaline (10^{-6} M), a β-adrenergic agonist, produced an eightfold increase in pinealocyte cyclic AMP and a doubling of cyclic GMP. Addition of 4-β-phorbol 12-myristate 13-acetate (PMA) or 4-β-phorbol 12,13-dibutyrate (PDBu), activators of protein kinase C[15], together with isoprenaline, potentiated the cyclic AMP, but not the cyclic GMP, response (Fig. 1). The maximal increase in cyclic AMP was comparable to that seen with noradrenaline, a mixed α- and β-adrenergic agonist. The concentrations of PMA and PDBu producing half-maximal potentiation were approximately 5 and 50 nM respectively. These concentrations are similar to the reported half-maximal concentrations necessary to activate protein kinase C[16]. 4-α-Phorbol 12,13-didecanoate (PDD), which does not activate protein kinase C, had no potentiating effect (Fig. 1). Addition of these phorbol esters alone produced no elevation in cyclic AMP.

An examination of the time course of the cyclic AMP response to isoprenaline (10^{-6}M) or isoprenaline plus PMA (10^{-7} M) indicated that potentiation was present after 1 min, reached a peak by 10 min and was still elevated after 1 h (Fig. 2). The small potentiation of the cyclic GMP response is probably an artefact caused by the cross-reactivity of the cyclic GMP antisera.

Measurement of protein kinase C activity in pinealocytes revealed a very high activity (42,000 pmol ^{32}P incorporated per 3 min per mg protein) when compared with most tissue[17,18] including neural tissues (Table 1). Treatment of cells with either phenylephrine (10^{-6} M) or PMA (10^{-7} M) caused a rapid decrease in cytosolic protein kinase C activity with a corresponding increase in membrane-associated activity. It has been suggested that the redistribution of this protein kinase activity may be an important, early event in the action of phorbol esters, perhaps enabling the enzyme to gain access to physiological substrates[19].

The physiological activator of protein kinase C is thought to be diacylglycerol, which is transiently formed from the breakdown of membrane phosphatidylinositol[20]. Noradrenaline, acting through α_1-adrenoceptors, is known to stimulate this process in the pineal gland[21,22]. A synthetic diacylglycerol analogue, 1-oleoyl 2-acetyl glycerol (OAG), can also activate C kinase[23] and furthermore is able to cross the cell membrane. OAG (12.5–50 μg ml^{-1}) potentiated the stimulation of cyclic AMP produced by isoprenaline (10^{-6} M) (Fig. 3). No effect of OAG alone was observed. A small but significant potentiation of the cyclic GMP response to isoprenaline cannot be completely explained by the cross-reactivity of the cyclic GMP antisera.

These studies clearly show that phorbol esters and OAG, both of which activate protein kinase C, mimic the action of α_1-agonists on pinealocytes and potentiate β-adrenergic stimulation of cyclic AMP content. In addition, protein kinase C is rapidly redistributed from the cytosol into membranes not only in response to PMA[19], but also by treatment of the intact cells with an α_1-agonist, phenylephrine. These findings, together with earlier evidence that postsynaptic α_1-adrenoceptors mediate a rapid increase in membrane phosphatidylinositol turnover in pinealocytes[21,22], suggest that activation of protein kinase C is involved in the α_1-adrenergic amplification of β-adrenergic stimulation of cyclic AMP accumulation in this cell. Presumably, diacylglycerol generated by noradrenaline-mediated phosphatidylinositol turnover interacts with protein kinase C in the

Table 1 Effect of phenylephrine or PMA treatment of pinealocytes on cytosolic and membrane-bound protein kinase C activity

Expt no.	Pretreatment	Protein kinase C activity (pmol ^{32}P incorporated per 3 min per 5×10^6 cells)	
		Cytosol	Membranes
1	Water	13,750	1,000
	Phenylephrine (10^{-6} M)	8,725	5,160
2	Vehicle	13,248	1,395
	PMA (10^{-7} M)	6,286	3,164

Pinealocytes (5×10^6) were treated with phenylephrine (10^{-6} M) for 1 min or with PMA (10^{-7} M) for 15 min. Cells were pelleted (1,000*g*, 1 min), washed, then lysed in water (250 μl) containing $CaCl_2$ (10^{-5} M) by brief (5-s) sonication (expt 1) or by repeated aspiration into a syringe through a fine (27-gauge) needle (expt 2). Membrane and cytosol were separated (12,000*g*, 1 min at 4 °C) and 0.5 ml of buffer A (20 mM Tris-HCl, *p*H 7.5, 2 mM EDTA, 0.5 mM EGTA, 2 mM phenylmethylsulphonyl fluoride) was added to each preparation. Cytosol was applied to a DE-52 cellulose column (0.5 × 1 cm) equilibrated with buffer A. The column was washed with 8 volumes of buffer A, and the enzyme eluted with 0.08 M NaCl in buffer A. Membranes were solubilized with Nonidet P-40 (1%) by gentle mixing for 30 min at 4 °C. The preparation was centrifuged (Beckman Microfuge, 10,000*g*, 2 min at 4 °C) and the detergent-solubilized preparation applied to a DE-52 cellulose column and eluted as described for cytosol. Protein kinase C activity was assayed in triplicate as described previously with 0.75 mM Ca^{2+}, in the presence and absence of 24 μg phosphatidylserine, and 1.6 μg diolein[17,30]. Protein kinase C activity was determined by subtracting the amount of ^{32}P incorporated into histone H1, in the absence of added phospholipids, from the amount incorporated in the presence of phospholipids. Protein kinase C activity is expressed as pmol ^{32}P incorporated per 3 min per total cytosolic and membrane fraction from 5×10^6 cells.

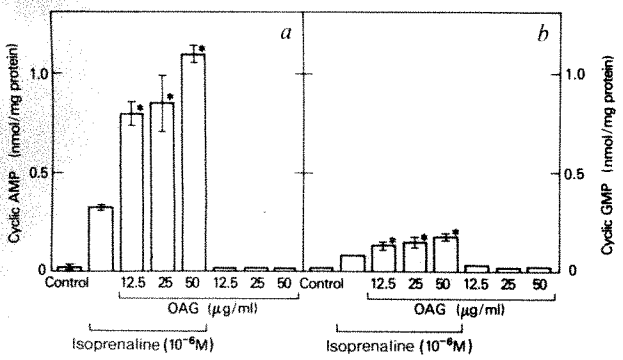


Fig. 3 Potentiation of β-adrenergic cyclic nucleotide responses by 1-oleoyl, 2-acetyl glycerol (OAG). OAG (10 mg ml^{-1} in chloroform) was dried under N_2, then suspended in dimethyl sulphoxide (1%) by brief, vigorous sonication on ice immediately before use. OAG (a gift from Dr Y. Nishizuka) was added as ×25 to ×100 concentrated stock suspension to give the final concentrations indicated. Pinealocytes (10^5 cells per tube) were pelleted (1,000g, 1 min) and resuspended in DMEM without fetal calf serum, then treated for 5 min with OAG alone or in combination with (−)isoprenaline. Each bar represents the mean ±s.e.m. of three samples. * Significantly different from isoprenaline alone, $P < 0.05$ using Duncan's multiple range test.

presence of membrane phospholipids and Ca^{2+} transiently to activate and stabilize the enzyme in association with the membrane. This activation of protein kinase C may result in the rapid phosphorylation of a component of the system synthesizing (β-adrenoceptor, guanine nucleotide binding proteins, adenylate cyclase catalytic subunit ?) or degrading (phosphodiesterase ?) cyclic AMP, an important intracellular messenger.

Apparently a different mechanism mediates the α_1-adrenergic amplification of the β-adrenergic cyclic GMP response. Unlike cyclic AMP, the accumulation of pineal cyclic GMP in response to noradrenaline is Ca^{2+}-dependent[24]. Conceivably the α_1-adrenergic amplification of the cyclic GMP response requires not only protein kinase C activation but also an elevation of intracellular Ca^{2+}. This may explain the significant potentiation of the cyclic GMP response by OAG; OAG would be expected to be rapidly metabolized to the corresponding phosphatidic acid[23], which may then increase intracellular Ca^{2+}. Inositol trisphosphate[25] or phosphatidic acid[26] generated as a result of an increased phosphatidylinositol turnover may be physiological mediators of this action of α_1-adrenoceptor stimulation. Alternatively, guanylate cyclase may be stimulated by arachidonic acid or a metabolite[27].

Finally, these studies are of special interest because, to our knowledge, they are the first to describe a synergistic interaction between these intracellular signalling systems. Previous work has generally suggested that activation of protein kinase C may reduce neurotransmitter or hormone-dependent cyclic AMP signals (for example, see refs 28, 29). Clearly, this is not the case in all cell types.

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1. Klein, D. C., Auerbach, D. A., Namboodiri, M. A. A. & Wheler, G. H. T. in *The Pineal Gland: Anatomy and Biochemistry* (ed. Reiter, R. J.) 199–227 (CRC Press, Boca Raton, Florida, 1981).
2. Klein, D. C. & Weller, J. L. *Science* **169**, 1093–1095 (1970).
3. Deguchi, T. *Molec. Pharmac.* **9**, 184–190 (1973).
4. Klein, D. C., Buda, M. J., Kapoor, C. L. & Krishna, G. *Science* **199**, 309–311 (1978).
5. Zatz, M., Kebabian, J. W., Romero, J. A., Lefkowitz, R. J. & Axelrod, J. *J. Pharmac. exp. Ther.* **196**, 714–722 (1976).
6. Sugden, D. & Klein, D. C. *Endocrinology* **114**, 435–440 (1984).
7. Klein, D. C., Sugden, D. & Weller, J. L. *Proc. natn. Acad. Sci. U.S.A.* **80**, 599–603 (1983).
8. Sugden, D., Weller, J. L., Klein, D. C., Kirk, K. L. & Creveling, C. R. *Biochem. Pharmac.* **33**, 3947–3950 (1984).
9. Auerbach, D. A., Klein, D. C., Woodard, C. & Auerbach, G. D. *Endocrinology* **108**, 559–567 (1981).
10. Klein, D. C., Auerbach, D. A. & Weller, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4625–4629 (1981).
11. Vanecek, J., Sugden, D., Weller, J. L. & Klein, D. C. *Endocrinology* (in the press).
12. Buda, M. & Klein, D. C. *Endocrinology* **103**, 1483–1493 (1978).
13. Harper, J. F. & Brooker, G. J. *Cyclic Nucleotide Res.* **1**, 207–218 (1975).
14. Bradford, M. M. *Analyt. Biochem.* **72**, 248–254 (1976).
15. Castagna, M. *et al. J. biol. Chem.* **257**, 7847–7851 (1982).
16. Kraft, A. S., Anderson, W. B., Cooper, H. L. & Sando, J. J. *J. biol. Chem.* **257**, 13193–13196 (1982).
17. Kuo, J. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 7039–7043 (1980).
18. Minakuchi, R., Takai, Y., Yu, B. & Nishizuka, Y. *J. Biochem., Tokyo* **89**, 1651–1654 (1981).
19. Kraft, A. S. & Anderson, W. B. *Nature* **301**, 621–623 (1983).
20. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. & Nishizuka, Y. *J. biol. Chem.* **255**, 2273–2276 (1980).
21. Berg, G. R. & Klein, D. C. *J. Neurochem.* **19**, 2519–2532 (1972).
22. Smith, T. L., Eichberg, J. & Hauser, G. *Life Sci.* **24**, 2179–2184 (1979).
23. Kaibuchi, K., Sano, K., Hoshijima, M., Takai, Y. & Nishizuka, Y. *Cell Calcium* **3**, 323–335 (1982).
24. O'Dea, R. F. & Zatz, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3398–3402 (1976).
25. Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. biol. Chem.* **259**, 3077–3081 (1984).
26. Ohsako, S. & Deguchi, T. *J. biol. Chem.* **256**, 10945–10948 (1981).
27. Snider, R. M., McKinney, M., Forray, C. & Richelson, E. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3905–3909 (1984).
28. Nishizuka, Y. *Nature* **308**, 593–698 (1984).
29. Anderson, W. B., Estival, A., Tapiovaara, H. & Gopalakrishna, R. *Adv. Cyclic Nucleotide Res.* (in the press).
30. Kraft, A. S. & Anderson, W. B. *J. biol. Chem.* **258**, 9178–9183 (1983).

Human granulocyte–macrophage colony-stimulating factor is a neutrophil activator

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The polymorphonuclear leukocyte (PMN), or neutrophil, is the major host defence cell protecting the body against invasion by bacteria and fungi. Products of oxidative metabolism mediate PMN microbicidal and tumoricidal activity[1] but the mechanisms by which these pathways become activated are not well understood. We have previously described a human granulocyte–macrophage colony-stimulating factor (GM-CSF) of relative molecular mass (M_r) 22,000 that also inhibits neutrophil motility (NIF-T activity)[2]. Because of its direct action on granulocytes, this lymphokine is a candidate for a neutrophil-activating factor. We have studied the effect of GM-CSF/NIF-T on superoxide anion generation in response to the bacterial chemo-attractant *N*-formylmethionyl-leucylphenylalanine (f-MLP)[3], and report here that PMNs preincubated with either purified natural GM-CSF or biosynthetic (recombinant) GM-CSF showed increased (as much as fourfold) superoxide anion production in response to f-MLP. These results indicate that human GM-CSF is a neutrophil-activating factor.

A neutrophil migration-inhibition factor from T lymphocytes (NIF-T) produced by a human T-lymphoblast cell line (Mo) was characterized as an acidic glycoprotein of M_r 22,000 (22K) with specificity for binding PMN plasma membranes[2,4–6]. Purified NIF-T was shown to have colony-stimulating activity, and expression of a cDNA for human GM-CSF demonstrated that the gene product has NIF-T activity[2], confirming the identity of these two proteins.

In the experiments reported here, we examined the effect of GM-CSF on superoxide anion production by neutrophils, measured as superoxide dismutase-inhibitable cytochrome *c* reduction[7]. PMNs incubated with Mo-conditioned medium alone for 2 h did not produce measurable superoxide anion. In 10 separate experiments, a mean (±s.e.m.) of 2.1 ± 0.2 nmol superoxide anion per min per 10^6 PMNs was produced over 4 min in the presence of 10^{-8} M f-MLP. However, when PMNs were preincubated for 2 h with Mo-conditioned medium before addition of f-MLP, superoxide anion production was increased to a mean of 7.0 ± 0.4 nmol per min per 10^6 PMNs measured over 4 min. The results of a representative experiment are shown in Fig. 1.

The degree of enhancement of f-MLP-induced oxidative metabolism was dependent on the length of time for which the PMNs

were incubated with Mo-conditioned medium (Fig. 2). A maximum (fourfold) increase in cytochrome *c* reduction (measured over 5 min) occurred when PMNs were incubated with Mo-conditioned medium for 2 h before addition of f-MLP (Fig. 2).

GM-CSF was isolated sequentially from Mo-conditioned medium by lectin affinity chromatography, gel filtration chromatography and reverse-phase HPLC, with co-purification of GM-CSF[2] and neutrophil-activating factor (NAF) activities (data not shown). GM-CSF isolated by HPLC was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Analysis of eluates from gel slices showed CSF and NAF activities corresponding to a 22K protein (Fig. 3). The NH_2-terminal amino-acid sequence of this protein is identical to that predicted by the nucleotide sequence of the human GM-CSF cDNA clone[2].

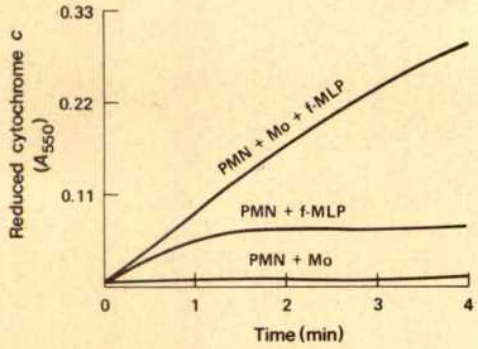


Fig. 1 Effect of Mo-conditioned medium on f-MLP-induced PMN oxidative metabolism.

Methods. PMNs were isolated from peripheral blood of healthy subjects by Ficoll–Hypaque gradient centrifugation, and red blood cells were removed by 6% dextran sedimentation and 30-s hypotonic lysis. PMNs (5×10^6 ml^{-1}) were suspended in Hanks' buffered salt solution (HBSS) (without phenol red) containing 5% fetal calf serum (FCS), and 0.4-ml aliquots containing 2×10^6 PMNs were incubated for 2 h at 37 °C with 0.040 ml of medium with 5% FCS alone or Mo-conditioned medium. After incubation, 2 ml of cytochrome *c* (1.2 mg ml^{-1} in HBSS with 5% FCS) were added to each cell suspension, mixed, and the contents divided equally into two tubes. Superoxide dismutase (0.010 ml of 2 mg ml^{-1}) was added to one of the tubes, and f-MLP was added as 1% of the volume to both tubes at a final concentration of 10^{-8} M. The cell suspensions were transferred immediately to disposable 1-ml cuvettes, and superoxide dismutase-inhibitable cytochrome *c* reduction was measured at 550 nm at 37 °C for 5 min in a Varian DMS 90 dual beam spectrophotometer. Absorbance was recorded every 5 s with a computer-assisted program. The data are expressed as the difference between results for Mo-conditioned medium and for medium alone.

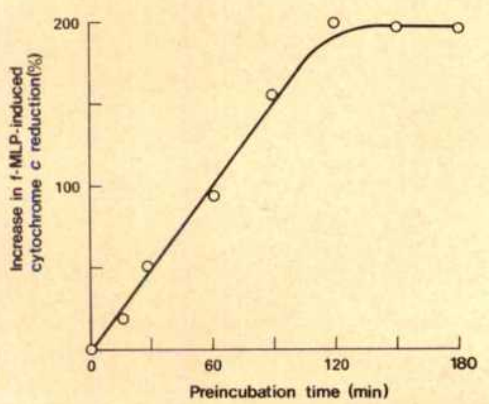


Fig. 2 Effect of length of preincubation with Mo-conditioned medium on f-MLP-induced PMN oxidative metabolism. Methods were the same as for Fig. 1 except that PMNs were preincubated for various periods of time with Mo-conditioned medium that titred 1:200 after 2 h of incubation. The data are expressed as percentage increase in mean A_{550} over 5 min after addition of f-MLP.

GM-CSF has been expressed in COS monkey cells transfected with a molecular clone of the human gene obtained independently from a cDNA expression library prepared from Mo mRNA[2,8]. Such biosynthetic recombinant GM-CSF did not directly increase PMN oxidative metabolism. In 10 separate experiments, PMNs incubated with conditioned medium from mock-transfected COS cells produced a mean (±s.e.m.) of 2.5 ± 0.3 nmol superoxide anion per min per 10^6 PMNs, measured over 4 min, in response to f-MLP. However, an average of 8.4 ± 0.7 nmol superoxide anion per min per 10^6 PMNs was produced over 4 min in response to f-MLP when PMNs were

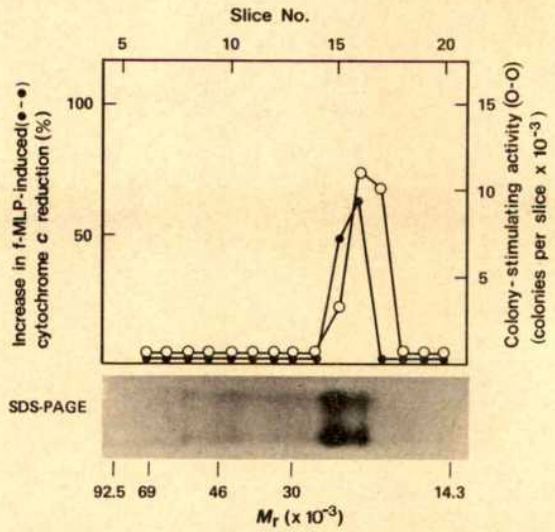


Fig. 3 Analysis of HPLC-purified CSF and NAF by SDS-PAGE. Ten litres of serum-free Mo-conditioned medium were concentrated and processed as described previously[2]. Briefly, the proteins precipitated with 80% ammonium sulphate were dialysed and applied to a lentil lectin–Sepharose 4B column, washed, and eluted with 0.5 M α-methyl-D-mannoside. The eluted glycoproteins were fractionated on an Ultrogel AcA 44 column, and fractions with peak GM-CSF activity were pooled and further fractionated by reverse-phase HPLC using a Vydac C-18 column. Protein was eluted with a gradient of acetonitrile containing 0.1% trifluoroacetic acid. HPLC fractions were evaporated to dryness, reconstituted and assayed for NIF-T, CSF and NAF activities. A fraction previously shown to contain GM-CSF was assayed and found to have NAF activity; this fraction was subjected to SDS-PAGE in a 10% polyacrylamide gel[2]. An aliquot of the sample was iodinated, fractionated with the unlabelled protein as a tracer, then analysed in parallel lanes in the presence and absence of 2-mercaptoethanol. The lane containing the unlabelled protein was sliced, eluted and assayed for NAF activity as well as NIF-T and CSF[2,10,11]. The results obtained for NIF-T and CSF have been published previously[2].

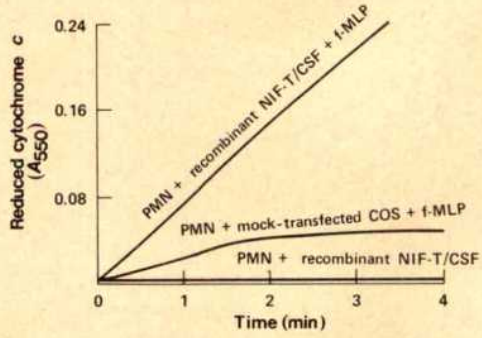


Fig. 4 Effect of biosynthetic recombinant GM-CSF on f-MLP-induced PMN oxidative metabolism. Computer-generated graph of measurements made at 5-s intervals. Methods as for Fig. 1, except that PMNs were preincubated with conditioned medium from monkey COS cells containing GM-CSF cloned[8] in the expression vector p91023(B) and with medium from mock-transfected COS cells.

preincubated with recombinant GM-CSF. (Figure 4 shows the results of a representative experiment.) Furthermore, GM-CSF purified to homogeneity from COS-conditioned medium by gel filtration chromatography and HPLC had neutrophil-activating activity. Control experiments using buffers, medium and conditioned medium from mock-transfected COS cells, and other HPLC fractions, eliminated the possibility that endotoxin contamination was responsible for these results. Also, the titres of NIF-T, CSF and NAF activities were the same in Mo-conditioned medium (1:200) and conditioned medium from COS cells transfected with the cloned GM-CSF gene (1:10,000).

These studies demonstrate that GM-CSF enhances oxidative metabolism of PMNs in response to the chemo-attractant f-MLP. Our results with this human lymphokine appear to correspond to those described for a murine CSF which has been shown to enhance granulocyte antibody-dependent cytotoxicity[9]. T-cell regulation of neutrophil oxidative metabolism by lymphokines may have an important role in neutrophil-mediated microbicidal and tumoricidal activity as well as in the pathogenesis of immune-mediated inflammatory diseases.

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1. Babior, B. M. *New Engl. J. Med.* **298**, 659-668, 721-725 (1978).
2. Gasson, J. C. *et al. Science* **226**, 1339-1342 (1984).
3. English, D., Roloff, J. S. & Lukens, J. N. *J. Immun.* **126**, 165-171 (1981).
4. Weisbart, R. H., Golde, D. W., Spolter, L., Eggena, P. & Rinderknecht, H. *Clin. Immun. Immunopath.* **14**, 441-448 (1979).
5. Weisbart, R. H. *et al. Clin. Immun. Immunopath.* **22**, 408-418 (1982).
6. Weisbart, R. J. *et al. J. Immun.* **128**, 457-462 (1982).
7. Rosen, H. & Klebanoff, S. J. *J. clin. Invest.* **58**, 50-60 (1976).
8. Wong, G. G. *et al. Science* (in the press).
9. Lopez, A. F. *et al. J. Immun.* **131**, 2983-2988 (1983).
10. Weisbart, R. H. & Mickey, M. R. *J. immun. Meth.* **16**, 269-281 (1977).
11. Cline, M. J. & Golde, D. W. *Nature* **277**, 177-181 (1979).

Levels of c-*myc* oncogene mRNA are invariant throughout the cell cycle

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The steady-state messenger RNA levels of several genes increase when cells are stimulated to proliferate[1-4]. The transcripts from one such gene, the proto-oncogene c-*myc*, increase approximately 20-fold shortly after cells are stimulated to proliferate and then decline before the onset of DNA synthesis[1,5]. It has been inferred from these data that expression of c-*myc* may be specific to the G_1 portion of the cell cycle[5]. Alternatively, this transient increase in c-*myc* mRNA following the stimulation of quiescent cells could be the result of an activational event that renders the cells competent to enter the cell cycle. To distinguish between these possibilities, we performed experiments to determine whether the amount of c-*myc* mRNA fluctuates during the cell cycle in cells that are under constant stimulation to proliferate. Although c-*myc* mRNA does undergo a transient increase within 2 h of serum stimulation of quiescent serum-deprived cells, our results show that the level of c-*myc* mRNA is constant throughout the cell cycle and does not diminish in density-arrested cells maintained in the presence of serum growth factors. In contrast to c-*myc*, the mRNA levels of two other genes whose expression has been associated with cellular proliferation[6-8] do show consistent variations within the cell cycle. Both thymidine kinase (TK) and histone 2b (H2b) mRNA levels increase during S phase in continuously growing cells and decrease when cell replication ceases in density-arrested cultures. Therefore, the transient increase in c-*myc* transcription following the activation of quiescent cells is not due to the type of cell cycle-dependent regulation characteristic of the TK and H2b genes.

To distinguish events which occur during the activation of quiescent cells to proliferate from events regulated within the cell cycle, we separated cells in exponentially growing cultures by counterflow centrifugation, a technique which fractionates cells primarily on the basis of size[9,10]. Because cells undergo a linear increase in volume as they progress through the cell cycle[9], a non-synchronized population of growing cells can be separated by size into subpopulations representing progressive stages of the cell cycle. This method avoids the perturbations in cellular metabolism which are inherent in experiments involving either chemical arrest or deprivation of essential growth factors[11]. To analyse events within the cell cycle, 10^8 exponentially growing cells were loaded into a counterflow centrifuge and separated into six subpopulations of increasing mean cell volume (Fig. 1). To verify that an effective cell cycle separation had occurred, the cells were stained with propidium iodide and the DNA-staining profile analysed on a fluorescence-activated cell sorter (FACS). The average fluorescence per cell of each fraction was calculated and expressed as a multiple of the diploid cell peak. Each fraction was then assigned an average ploidy value ranging from $2n$ for G_1 cells to $4n$ for G_2/M cells.

Two types of exponentially growing cell cultures were analysed in this way. MSB-1, a transformed chicken T-cell line with structurally normal c-*myc* alleles[12], was grown continuously in suspension culture. Chicken embryo fibroblasts (CEF) were dissected from the developing breast muscle of 11-day-old embryos and grown at low density on plastic Petri dishes[7]. Total RNA from cell cycle-dependent subpopulations was isolated using guanidinium isothiocyanate[13]. Ribosomal RNA increased in direct proportion to cell volume as cells progressed from G_1 to G_2/M; because of this, the levels of ribosomal RNA were equalized (Fig. 2) to avoid the measurement of mRNA variations attributable only to increases in cell volume during growth. Northern blots were prepared from the equalized RNA samples and hybridized sequentially with probes specific for the c-*myc* and TK genes. The blots were also hybridized to probes for the β-actin gene, which encodes an essential structural protein required for cell integrity[14] and therefore should be transcribed in all viable cells, and the gene for H2b, which has been shown previously to be cell cycle-regulated[15]. As shown in Fig. 2, the level of c-*myc* mRNA was equivalent in all of the cell-cycle dependent subpopulations of both CEF and MSB-1 cells. The c-*myc* mRNA levels as measured by scanning densitometry varied <20% between any two fractions in an individual experiment; this was a consistent finding in several experiments involving both cell types. Levels of β-actin mRNA were also constant in all subpopulations. In contrast, although TK mRNA was present throughout the cell cycle during exponential growth, a two- to sixfold increase in the level of this transcript was found in subpopulations enriched for S-phase cells. Similarly, the H2b mRNA level was 5-15 times greater in the subpopulations containing S-phase cells in both cell types. The quantitative variation in the cell-cycle expression of the H2b gene that we observed was comparable to that reported by others[15,16].

As an alternative approach to analysing the association of c-*myc* expression with cell proliferation, the levels of c-*myc* mRNA were examined as cells were shifted between exponential growth and growth arrest (Fig. 3*a*). In the continuous presence of serum growth factors, little difference in the levels of c-*myc* mRNA was observed between cultures in exponential growth and those that had ceased proliferation. This contrasts with a >10-fold reduction of TK mRNA in both CEF and MSB-1 cells as they stopped proliferating. When quiescent cells were

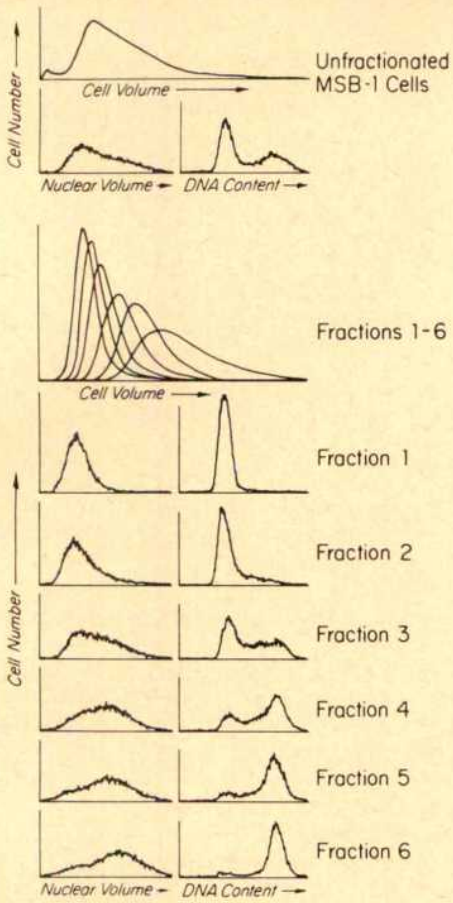


Fig. 1 Representative cell volume, nuclear volume and DNA content analysis of elutriated cell fractions. Exponentially growing MSB-1 cells were separated by counterflow centrifugation (elutriation) into six fractions of increasing cell size which were numbered 1–6 from smallest to largest. The recovery of the unfractionated cells in the six subpopulations was 93%. Cell volume was measured using an electronic cell counter/sizer. Nuclei were prepared by solubilization of cells with 0.5% NP40 in Tris-buffered saline containing 10 mM $MgCl_2$. Nuclear volume was determined by measuring narrow-angle light scatter on a FACS, and the average DNA content of cells from each fraction was determined by FACS analysis after staining of the nuclei with propidium iodide. *n* is the haploid DNA content of MSB-1 cells. Unfractionated cells gave a bimodal DNA curve characteristic of exponentially growing cells[9]. The early fractions from the elutriation contained cells with a 2*n* DNA content characteristic of G_1 cells. As cells of increasing size were recovered, the nuclear volume and average DNA content per cell increased. The final fraction contained almost exclusively cells with 4*n* DNA content, characteristic of G_2/M cells.

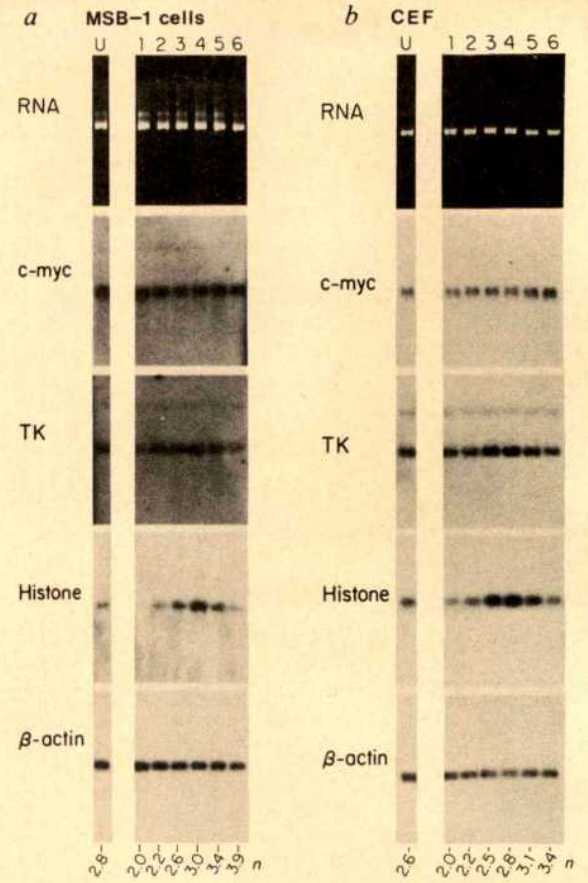


Fig. 2 The c-*myc*, TK, H2b and β-actin mRNA content of cell cycle-separated MSB-1 cells and CEF. Total RNA was prepared from the unfractionated population (U) and each of the subpopulations. The top panels of *a* and *b* show ethidium bromide staining of equal amounts of the RNA samples separated on a non-denaturing 1% agarose gel. These equalized RNA samples (2–5 μg depending on the experiment) were separated on 1% agarose/formaldehyde gels and blotted onto nitrocellulose. Filters were then hybridized with a ^{32}P-labelled 3.2-kilobase (kb) *Sst*I fragment containing the chicken c-*myc* gene[7], a 2.9-kb *Hin*dIII fragment containing a portion of the TK gene[7] (a gift from M. Wigler), a 0.59-kb *Hin*dIII fragment containing the 3′-untranslated region of the β-actin gene[14] (a gift from D. W. Cleveland) or a 2.3-kb *Hin*dIII/*Bam*HI fragment containing the H2b gene[24] (a gift of P. A. Krieg). Each filter was probed sequentially with several of these fragments to ensure that the hybridization observed represented true variations between samples rather than variations in sample preparation. Autoradiograms were exposed for 2–48 h and equivalent exposures compared. Relative molecular masses of the mRNAs that hybridized to each of the probes were similar to those published previously[7,14,24]. The additional band present on some TK and c-*myc* hybridizations was due to nonspecific hybridization to 28S ribosomal RNA and thus provides confirmation that equal amounts of RNA were transferred to the filters. Quantitation was performed by densitometry[1]. Numbers below the autoradiograms represent average haploid DNA content (*n*) of cells in each fraction.

recultured at lower density, both cell types resumed growth and the level of TK mRNA increased, while c-*myc* mRNA remained relatively constant. Again, the H2b mRNA levels paralleled those of TK. Although β-actin mRNA levels were constant in the presence of serum factors in MSB-1 cells, there did seem to be an increase in β-actin mRNA levels during exponential growth of CEF. Similar increases in β-actin mRNA levels have been reported when fibroblasts spread out in subconfluent culture conditions[17].

Figure 3*a* shows that c-*myc* expression is independent of the proliferative state of a cell. Others have shown that before cells can proliferate they must be activated by growth factors to a state where they are competent to enter the cell cycle[18]. Thus, previously described variations in levels of c-*myc* mRNA could be the result of this activation process, rather than the result of regulation during the proliferative cycle. Consistent with the reports of others using murine 3T3 cells[1,2,4], we find that serum-deprived cultures of both CEF and MSB-1 avian cells had lower amounts of steady-state c-*myc* mRNA relative to ribosomal RNA (data not shown). However, the steady-state levels of TK, H2b and β-actin mRNAs also decreased during serum deprivation. Although the cells remained viable and an increase in the levels of c-*myc* was observed on restimulation, it is difficult to be certain that these variations are anything more than a general decrease in steady-state mRNA due to sub-optimal culture conditions. Nevertheless, the response of serum-deprived CEF to the addition of serum growth factors did provide another demonstration of the fact that c-*myc* expression was regulated independently of TK and H2b gene expression. Figure 3*b* shows an experiment in which confluent CEF were serum-deprived for 3 days and then re-exposed to serum while still confluent. c-*myc* mRNA underwent a transient three- to fivefold increase in

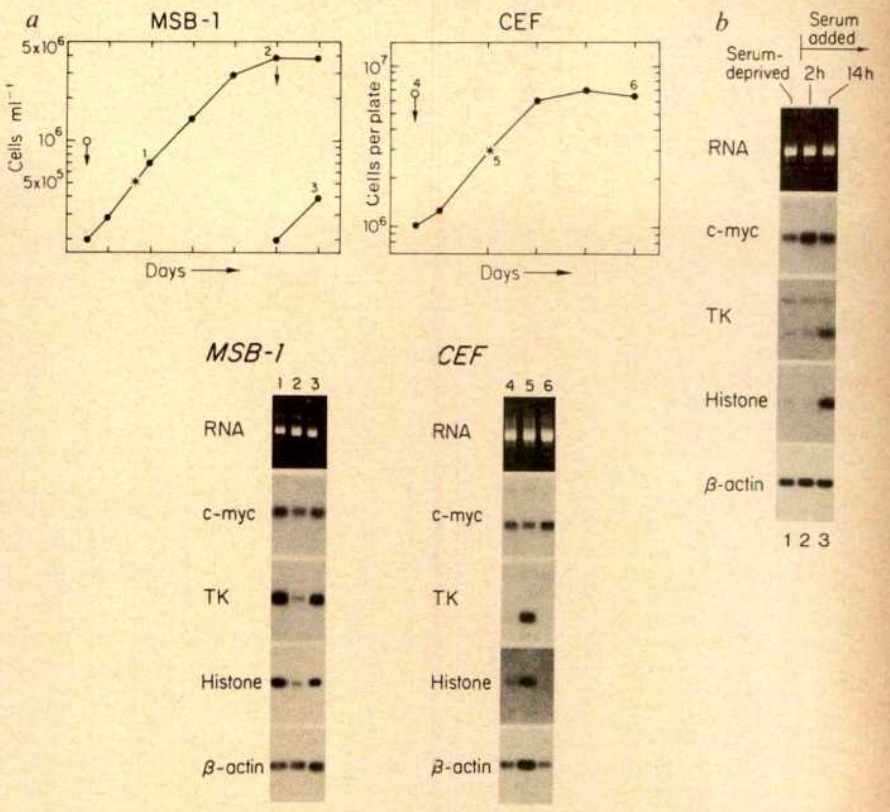


Fig. 3 *a*, Growth curves of MSB-1 cells and CEF. Exponentially growing MSB-1 cells were followed in culture until no further increase in cell number was observed. They were then recultured at a lower density and resumed exponential growth. Confluent CEF were recultured at a density of 1×10^6 per 150-mm plates and followed to confluency. Below the growth curves are autoradiograms of Northern blots prepared with equal amounts of RNA and hybridized with c-*myc*, TK, H2b and β-actin as described in Fig. 2 legend. For MSB-1, lane 1 represents RNA isolated from cells during initial exponential growth; lane 2, RNA from growth-arrested cells; and lane 3, RNA from cells after resumption of exponential growth. For CEF, lane 4 represents RNA isolated from confluent cells; lane 5, RNA from cells in exponential growth; and lane 6, RNA from cells after they have become confluent again. Cell viabilities as measured by trypan blue exclusion were >95% at each of the assay points for both CEF and MSB-1 cells. In both cell types, >40% of the cells were in S or G_2/M phase during exponential growth and <5% had greater than 2*n* DNA content during growth arrest. ↓ Indicates the point at which cells were recultured; * is the point on the growth curve at which cells were used for separation into cell cycle-dependent subpopulations using counterflow centrifugation, shown in Fig. 2. *b*, Effect of 3 days' culture of confluent CEF in the presence of medium containing 0.1% serum, followed by stimulation by addition of 6% serum to the culture. Total RNA was prepared before stimulation and 2 h and 14 h after stimulation. Top panel, ethidium bromide-stained gel showing the ribosomal RNAs after equalization; lower panels, autoradiograms of Northern blots of identical 1% agarose/formaldehyde gels hybridized with c-*myc*, TK, H2b and β-actin genes. In each panel, lane 1 is RNA isolated from cells before serum stimulation; lane 2 is RNA isolated from cells 2 h after stimulation; and lane 3 is RNA isolated from cells 14 h after stimulation.

expression 2 h after serum addition and then fell to near quiescent levels by 14 h. The TK and H2b mRNAs were barely detectable either before the addition of serum or 2 h after serum addition, but increased substantially by 14 h, the approximate time required for quiescent CEF to reach S phase following re-exposure to serum factors[18].

Finally, because the conditions in which cells are maintained *in vitro* may be different from the conditions in which cells proliferate *in vivo*, we examined the expression of c-*myc* and TK genes in cell cycle-dependent subpopulations of thymocytes (Fig. 4). Thymocytes proliferating *in vivo* had a wider size range than exponentially growing cells *in vitro*, and the first several subpopulations eluted from the counterflow centrifuge were made up exclusively of cells with 2*n* DNA. The rest of the subpopulations were composed of cells in progressively later stages of the cell cycle, comparable to the subpopulations obtained from CEF and MSB-1 cells. Figure 4 shows that the level of c-*myc* mRNA was slightly lower in the 2*n* thymocyte subpopulations but was constant in all subsequent fractions. The lower c-*myc* mRNA levels in the smaller 2*n* subpopulations correlated closely with the percentage of cells in these subpopulations which had the surface phenotype of mature T cells as assessed by monoclonal antibodies[19] (up to 30% of the cells in the subpopulation containing the smallest cells). Therefore, the 2*n* subpopulations are composed of both thymocytes in the G_1 phase of the cell cycle and small terminally differentiated T cells destined to leave the thymus[20]. Because terminally differentiated cells have been shown to contain low amounts of c-*myc* mRNA[4,21], the low level of c-*myc* mRNA in the 2*n* subpopulations presumably represents the average c-*myc* content of mature T cells and G_1 thymocytes. In contrast to c-*myc*, genes for H2b and TK were expressed only in the subpopulations containing S-phase cells; β-actin mRNA levels were constant in all fractions. Our results suggest that the *in vivo* expression of c-*myc*, H2b, TK and β-actin genes in proliferating thymocyte cells is similar to their expression in cells proliferating *in vitro*.

Previous reports[1,4,5] have shown that the c-*myc* mRNA levels of murine cells are low in quiescent serum-deprived cells and high in exponentially growing cells. Moreover, growth factors which cause cells to enter the cell cycle have been demonstrated to induce c-*myc* transcription[2]. It has also been shown that following removal of the growth factor responsible for the induction of proliferative competency, the c-*myc* mRNA level rapidly declines, and despite this, cells can proceed through the cell cycle they have already initiated[5]. These results could mean that c-*myc* is a cell-cycle-regulated G_1-specific transcript[5]. However, for the cells we have examined, the level of c-*myc* mRNA is constant throughout the cell cycle in the continuous presence of serum growth factors. This appears to be a general phenomenon, as we have obtained similar results in a variety of cell types in addition to those presented above, including murine BALB/c 3T3 cells[1], human HL60 cells[21] and avian BK25 cells[12] (data not shown). As this constant level of c-*myc* mRNA was seen in RNA samples containing equal amounts of ribosomal RNA, and the amount of ribosomal RNA increases as a function of cellular volume as cells progress though the cell cycle, c-*myc* must be transcribed throughout the cell cycle. In the accompanying paper[22], data are presented which show that c-*myc* protein is also translated as a fixed percentage of total protein synthesis throughout the cell cycle of both avian and human cells. Based on these results, we postulate that c-*myc* expression reflects the competency of cells to enter and progress through the cell cycle. In the presence of the appropriate growth factors, both proliferative competency and c-*myc* expression can be maintained irrespective of position in the cell cycle.

In contrast to our results with c-*myc*, the TK mRNA level did seem to vary within the cell cycle. TK gene expression has been linked previously to S phase by experiments showing an increase in TK enzymatic activity during DNA replication[6,23]. Our data demonstrate a similar association between increased TK mRNA levels and S phase. In a pattern of expression that is similar to that for H2b, TK mRNA levels increased during S phase,

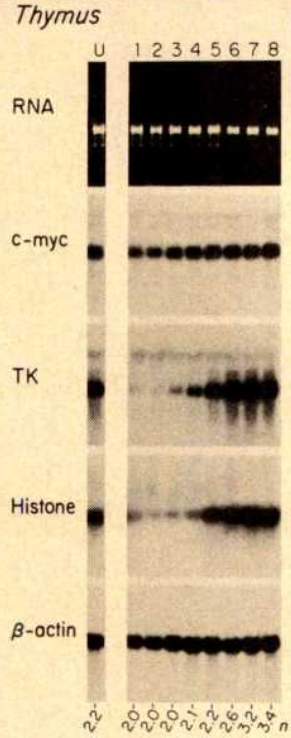


Fig. 4 The c-*myc*, TK, H2b and β-actin mRNA content of cell cycle-separated thymocytes. Numbers at the bottom of the lanes represent the average DNA content per cell (*n*). Thymocytes were prepared by gently teasing apart the thymuses of 6-week-old chickens. and filtering the cell suspension through nylon mesh to remove clumped cells. Contaminating non-lymphoid cells were removed by centrifuging the cell suspension over Ficoll-Hypaque. Greater than 95% of cells prepared in this manner stain positively with monoclonal antibodies specific for thymocyte antigens[19]. Cells were separated into subpopulations and analysed in a manner analogous to that shown in Fig. 1 except that two additional subpopulations were isolated at the beginning of the separations due to the wide distribution in size of thymocytes proliferating *in vivo*. Over 50% of the cells in the unfractionated population were isolated in the first three fractions, all of which were exlusively 2*n* in DNA content. Thereafter, the average DNA content and nuclear volume per cell in each fraction increased, indicating that a separation on the basis of cell cycle had occurred. Total RNA was prepared from the unfractionated thymocytes (U) of each of the subpopulations. Ribosomal RNA concentrations in all fractions were equalized and ethidium bromide staining of the equalized ribosomal RNAs, separated on a 1% agarose gel, is shown in the upper panel. Autoradiograms of Northern blots were prepared and hybridized with c-*myc*, TK, H2b and β-actin as described in Fig. 2 legend.

decreased as the number of replicating cells declined in culture, and was not expressed until 14 h after serum stimulation of quiescent cells. However, the variation of TK mRNA levels within the cell cycle of exponentially growing cells was not as large as the variation of H2b mRNA. There are several possible reasons for this. Unlike the H2b gene, expression of the TK gene may be only partially S-phase-dependent. For example, histone variants have been described in which transcription is induced at the start of DNA synthesis but is not completely repressed following S phase[16]. Alternatively, the half-life of TK mRNA may be greater than that of H2b, possibly because of the lack of polyadenylation of histone mRNA[8].

Taken together, these data define two broad categories of mRNA expression with respect to the cell cycle. Some genes, such as those for TK and H2b, are expressed at specific points in the cell cycle and can be said to be cell-cycle-regulated. A second class of genes, represented by c-*myc*, are expressed in cells capable of proliferating and are transcribed continuously throughout the cell cycle.

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1. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. *Cell* **35**, 603–610 (1983).
2. Greenberg, M. E. & Ziff, E. B. *Nature* **311**, 433–438 (1984).
3. Goyette, M., Petropoulos, C. J., Shank, P. R. & Fausto, N. *Molec. cell. Biol.* **4**, 1493–1498 (1984).
4. Campisi, J. *et al. Cell* **36**, 241–247 (1984).
5. Kelly, K., Cochran, B., Stiles, C. & Leder, P. *Curr. Topics Microbiol. Immun.* **113**, 117–126 (1984).
6. Johnson, L. F., Rao, L. G. & Muench, A. J. *Expl Cell Res.* **138**, 79–85 (1982).
7. Groudine, M. & Casimir, C. *Nucleic Acids Res.* **12**, 1427–1446 (1984).
8. Sittman, D. B., Graves, R. A. & Marzluff, W. F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1849–1853 (1983).
9. Alterman, R.-B. M. *et al. Molec. cell. Biol.* **4**, 123–132 (1984).
10. Thompson, C. B. *et al. J. Immun.* **133**, 2333–2342 (1984).
11. Grdina, D. J. *et al. Cell Tissue Kinetics* **17**, 223–236 (1984).
12. Schubach, W. & Groudine, M. *Nature* **307**, 702–708 (1984).
13. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
14. Cleveland, D. W. *et al. Cell* **20**, 95–105 (1980).
15. Osley, M. A. & Hereford, L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7689–7693 (1982).
16. Zweidler, A. in *Histone Genes: Structure, Organization & Regulation* (eds Stein, G. S., Stein, J. L. & Marzluff, W. F.) 339–371 (Wiley, New York, 1984).
17. Farmer, S. R., Wan, K. M., Ben-Ze'ev, A. & Penman, S. *Molec. cell. Biol.* **3**, 182–189 (1983).
18. Pledger, W. J., Stiles, C. D., Antoniades, H. N. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4481–4485 (1977).
19. Chen, C. H., Chanh, T. C. & Cooper, M. D. *Eur. J. Immun.* **14**, 385–391 (1984).
20. Sekaly, R. P., Ceredig, R. & MacDonald, H. R. *J. Immun.* **131**, 1085–1089 (1983).
21. Westin, E. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2490–2494 (1982).
22. Hann, S. R., Thompson, C. B. & Eisenman, R. N. *Nature* **314**, 366–369 (1985).
23. Schlosser, C. A., Steglich, C., DeWet, J. R. & Scheffler, I. E. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1119–1123 (1981).
24. Krieg, P. A. & Melton, D. A. *Nature* **308**, 203–206 (1984).

c-*myc* oncogene protein synthesis is independent of the cell cycle in human and avian cells

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Several lines of evidence suggest a role for the *myc* oncogene in cell proliferation.[1–7] Most recently, mitogenic stimulation of quiescent lymphoid, fibroblast and epithelial cells has been demonstrated to lead to a sharp increase in c-*myc* RNA levels[8–10]. To determine how c-*myc* expression is linked to the cell proliferative cycle, we have used centrifugal elutriation to enrich for populations of avian and human cells at different stages of the cell cycle. Centrifugal elutriation is a counterflow centrifugation method that separates cells on the basis of volume, a parameter correlating well with progression through the cell cycle[11–14]. Using *myc*-specific anti-peptide antibodies, we show here that the synthesis, half-life and modification of c-*myc* proteins are constant throughout the cell cycle of normal and transformed cells.

Figure 1*a* (upper panel) shows distributions of cell volume, nuclear volume and DNA content for an exponentially growing population of K562 cells. The K562 line is derived from a human chronic myelogenous leukaemia[15] containing a chromosome 9 translocation involving the c-*abl* oncogene, but does not contain either amplified or translocated *myc* sequences[16]. These cells display the broad range of nuclear and cell volumes expected for an unsynchronized population of growing cells. In addition, the DNA content profile indicates the presence of cells in G_1, S and G_2/M phases of the cell cycle. Using centrifugal elutriation of this population of K562 cells, we have collected six subpopulations differing in cell volume (Fig. 1*a*, lower panel). Flow cytometric analysis of these fractions indicates that fraction 1 has a DNA content characteristic of cells in G_1, fractions 2 and 3 contain cells in G_1 with increasing numbers of S-phase cells, fractions 4 and 5 contain primarily S-phase cells with some G_1 and G_2/M cells, respectively, and fraction 6 contains predominantly G_2/M cells. The increase in nuclear and cellular volume observed during progression through the cell cycle is paralleled by an increase in both the quantity of ribosomal RNA and amino-acid incorporation, to levels in G_2/M cells more than twice those found in G_1 cells (data not shown).

To determine the level of c-*myc*-encoded protein synthesis, K562 cells were pulse-labelled for 20 min with ^{35}S-methionine before elutriation. The labelled cells from each elutriator fraction were lysed and sonicated in buffer containing detergents. Because incorporation of the labelled amino acid increased in direct proportion to cell volume during progression through the

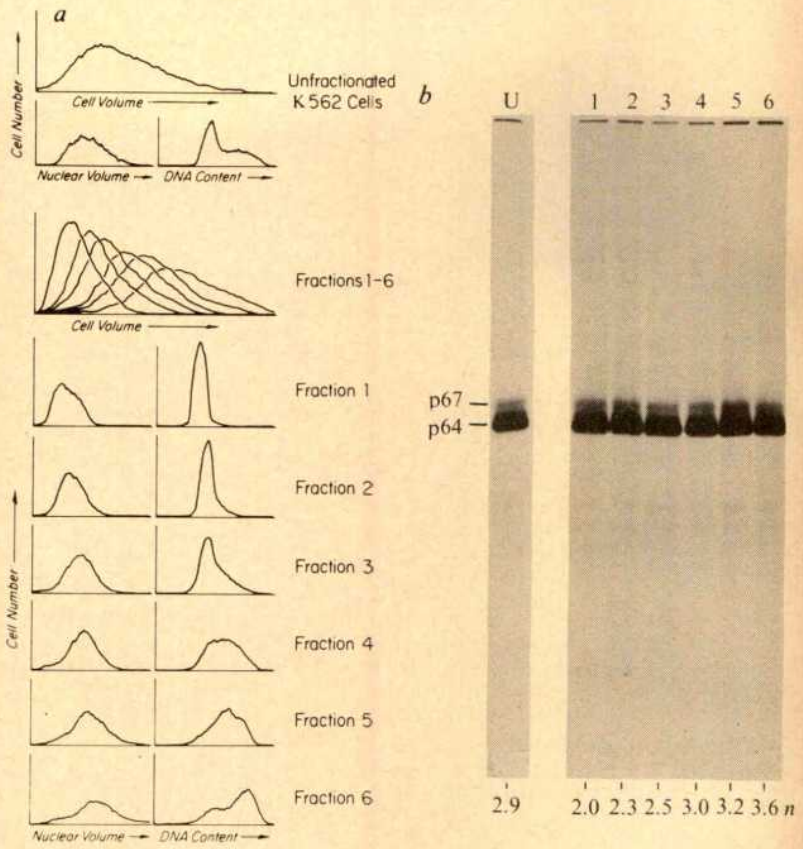


Fig. 1 *a*, Representative cell volume, nuclear volume and DNA content analysis of elutriated cell fractions. *b*, Analysis of c-*myc*-encoded proteins in cell cycle-separated K562 cells. *N* represents the haploid DNA content of K562 cells; U, unfractionated population.

Methods. *a*, An exponentially growing culture of the human chronic myelogenous leukaemia cell line K562 was suspended in Tris-buffered saline with 0.5 mM EDTA, 5% calf serum at 4 °C and separated by counterflow centrifugation into six fractions numbered 1–6 in order of increasing cell size. The recovery of the input unfractionated cells from the six subpopulations was 90%. Cell volume was measured using an electronic cell counter/sizer. Nuclear volume was determined by measuring narrow angle laser light scatter on a fluorescence-activated cell sorter (FACS) after preparation of nuclei by solubilization of cells with 0.5% NP40 in Tris-buffered saline containing 10 mM $MgCl_2$. The average DNA content of cells from each fraction was determined by FACS analysis after staining the nuclei with propidium iodide. *b*, The K562 cells (10^8 cells) shown in the elutriation fractions of *a* were first labelled with 3 mCi ^{35}S-methionine before elutriation. The elutriated subpopulations were lysed in Ab. buffer (0.01 M Tris-Cl *p*H 7.5, 0.05 M NaCl, 0.5% NP40, 0.5% deoxycholate, 0.5% SDS, 0.5% Trasylol, 1 mM phenylmethylsulphonyl fluoride, 10 mM iodoacetamide), followed by 30 s of sonication and centrifugation at 10,000*g* for 10 min. The lysates were adjusted to equal levels of incorporated label and brought to the same volume. Immunoprecipitation was as described previously using anti-hu-*myc* 12C (affinity-purified antibodies prepared against a synthetic peptide whose sequence corresponds to the 12 C-terminal amino acids of the human c-*myc* coding region)[17]. The immunoprecipitate was dissolved by boiling for 3 min in SDS electrophoresis sample buffer (5% SDS, 4% β-mercaptoethanol, 0.08 M Tris-Cl *p*H 6.8, 10% glycerol, 0.002% bromophenol blue) and electrophoresed on a discontinuous 10% SDS-polyacrylamide slab gel. A fluorograph of the dried gel is shown. M_rs were determined as previously described[17]; M_r markers were co-electrophoresed on all gels. Quantitation was carried out by scanning laser densitometry of fluorographs.

cell cycle (data not shown), we adjusted the amounts of lysate to normalize for equal amounts of incorporated label[17]. This allows an analysis of changes in c-*myc* protein levels that are not due simply to changes in cell mass during growth. The c-*myc* proteins were immunoprecipitated with an anti-peptide antibody reactive with the carboxy-terminus of human c-*myc* proteins (anti-hu-*myc* 12C)[17]. Figure 1*b* demonstrates that K562 cells synthesize the major and minor human c-*myc*-specific proteins p64 and p67 (respective relative molecular masses (M_rs) 64,000 and 67,000) as previously observed for cells with unrearranged c-*myc*,[17] and also that the levels of $p64/67^{myc}$ are constant (<25% maximum variation among the fractions) in the six cell fractions derived from different phases of the cell cycle. The short labelling time used in these experiments (20 min) coupled with the rapid elutriation time (45 min) at 4 °C implies that we are measuring primarily the rate of synthesis of *myc* proteins, which have a half-life of ~25 min at 37 °C[17]. Similar results to those shown in Fig. 1*b* were obtained when cell populations were labelled after elutriation (data not shown), indicating that the elutriation procedure is unlikely to be perturbing *myc* protein metabolism.

To find whether production of c-*myc* protein is different in transformed cells containing an altered c-*myc* allele, we also examined Manca, a human Burkitt's lymphoma cell line, which contains a translocation (t(8; 14)) of c-*myc* exons 2 and 3 into the immunoglobulin heavy-chain locus[18,19] and produces only the $p64^{myc}$ protein[17] (see Fig. 2*a*, lane U). When c-*myc* protein synthesis in Manca cells was assayed after fractionation by elutriation into cell cycle stages, as seen with K562 cells, the rate of synthesis of $p64^{myc}$ showed little variation (23% among the average G_1, S and G_2/M subpopulations) throughout the growth cycle when cell extracts were normalized to equal amounts of incorporated label (Fig. 2*a*).

We next attempted to generalize these observations by examining c-*myc* protein synthesis as a function of the cell cycle in normal and transformed avian cells. Accordingly, we labelled with ^{35}S-methionine: (1) secondary quail embryo fibroblast cells (QEF), (2) a Marek's disease virus-transformed chicken thymocyte line (MSB-1 cells) containing unrearranged c-*myc* (ref. 20) and (3) the chicken bursal lymphoma cell line BK25, containing a c-*myc* gene activated by insertion of a long terminal repeat[21]. The labelled cells were fractionated by elutriation, the lysates adjusted to equal amounts of incorporated label and avian c-*myc* proteins identified by immunoprecipitation with anti-peptide antiserum prepared against the carboxy-terminus of the MC29 v-*myc*-encoded protein (anti-v-*myc* 12C)[22]. The QEF (Fig. 2*b*) and MSB-1 cells (Fig. 2*c*) showed the avian $p59/62^{c\text{-}myc}$ proteins characteristic of cells possessing unaltered c-*myc* genes, while the BK25 bursal lymphoma line (Fig. 2*d*) showed high levels of only one of the c-*myc* proteins ($p59^{myc}$), similar to that previously reported for human tumour cells containing c-*myc* alterations[17]. The data again demonstrate no consistent change in the levels of synthesis of these c-*myc*-encoded proteins. A maximum of 20% variation was found among the BK25 fractions, with 30% variation among the MSB-1 fractions.

We also assayed for c-*myc* protein synthesis in a synchronized QEF population at various times after release from a double-thymidine block at the G_1/S boundary. Cells released into serum-containing medium doubled in cell number ~10 h after release, but levels of c-*myc* protein synthesis, except for a transient increase 1 h after release, did not change significantly throughout the 24-h QEF cell cycle (Fig. 2*e*). In addition, the G_1/S-blocked non-proliferating population had levels of c-*myc* protein synthesis equal to that of the mock-treated proliferating cells (compare Fig. 2*e*, lanes 0 and M). In a parallel experiment,

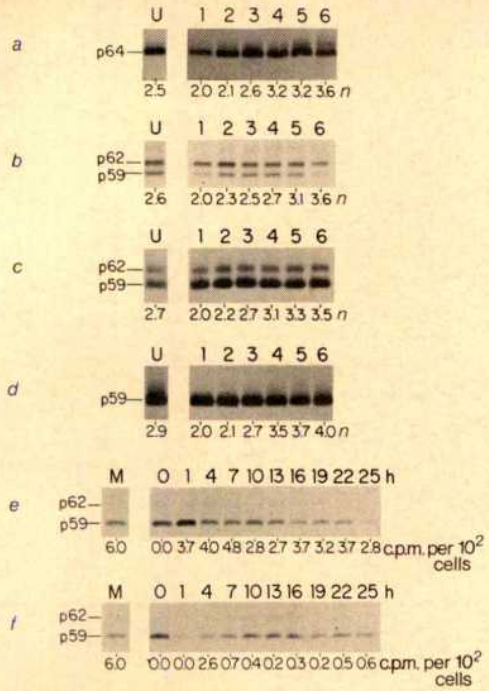


Fig. 2 *a–d*, Synthesis of c-*myc* proteins in human and avian cells separated into cell cycle subpopulations. Exponentially growing cells (10^8) were labelled for 20 min with 1–3 mCi ^{35}S-methionine before elutriation. Lysates from cell cycle subpopulations were adjusted to equal amounts of incorporated label and immunoprecipitated (*a*, anti-hu-*myc* 12C; *b–d*, anti-v-*myc* 12C (ref. 22)) as described in fig. 1 legend. *a*, Manca cell line, a human Burkitt's lymphoma cell line[18,19]; *b*, quail embryo fibroblast cells, secondary culture; *c*, MSB-1 cells, a chicken thymocyte line; *d*, BK25, a chicken cell line derived from a bursal lymphoma. Numbers below the autoradiograms represent average DNA content of cells in each fraction. U, unfractionated cells. *e*, *f*, Synthesis of c-*myc* proteins in chicken embryo fibroblast cells synchronized by double-thymidine block. Secondary cultures of chicken embryo fibroblasts (CEF) were grown in 100-mm dishes to 5×10^6 cells per dish and treated with 25 mM thymidine for 18 h. This was followed by growth in complete medium for 9 h and another 18-h incubation in complete medium containing 25 mM thymidine. Cells were labelled with 500 μCi ^{35}S-methionine per ml for 20 min before removal of the thymidine (0 time point) and at 3-h intervals after removal of the thymidine and incubation in growth medium containing 10% calf serum, 2% chick serum (*e*) or in growth medium lacking serum (*f*). Preparation of lysates and immunoprecipitation with anti-v-*myc* 12C were as described in Fig. 1 legend. Parallel cultures were labelled for 20 min with 5 μCi ^{3}H-thymidine per ml and aliquots of lysates were precipitated with trichloroacetic acid to determine incorporation of label into DNA as denoted by numbers below the lanes. Synchrony induction was confirmed by direct cell counting. Lanes M, mock-treated cultures. Only the relevant portions of the autoradiograms are shown. Lower band intensities in *e* (25 h) and *f* (1 h) lanes were due to sample loss during preparation of immunoprecipitates.

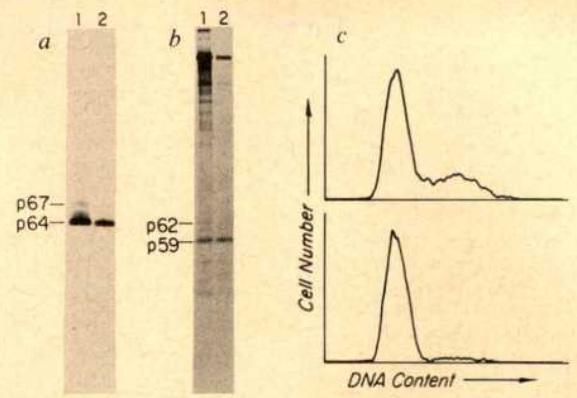


Fig. 3 Synthesis of c-*myc* proteins in quiescent and growing human and avian cells. *a*, K562 cells; *b*, QEF. Lanes 1, exponentially growing cells; lanes 2, quiescent cells. *c*, FACS analysis of the DNA contents of QEF cells in *b*. Upper panel, exponentially growing cells; lower panel, quiescent cells.
Methods. Human K562 cells were grown to saturation (10^6 cells per ml) and tertiary cultures of QEF were grown to confluency (2×10^7 cells per 100-mm dish) and incubated in normal growth medium for an additional 48 h. The cells were then incubated for a further 48 h in medium containing 0.1% serum (quiescent cells) and then labelled for 20 min with 500 μCi ^{35}S-methionine in medium lacking serum. Parallel cultures of non-serum-starved exponentially growing cells (4×10^5 ml^{-1} K562 cells; 10^7 per 100-mm dish QEF) were also maintained and labelled at the same time. FACS analysis indicated the presence of G_1, S and G_2/M cells in the exponentially growing populations. Lysis and immunoprecipitation with anti-v-*myc* 12C were as described in Fig. 1 legend.

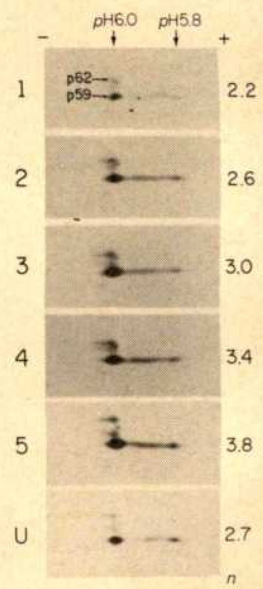


Fig. 4 Two-dimensional gel electrophoretic analysis of c-*myc* proteins synthesized in cell cycle-separated subpopulations of MSB-1 cells. Numbers on the left-hand side refer to the cell fractions; U, unfractionated cells; *n* represents the haploid DNA content.
Methods. Chicken MSB-1 cells (10^8 cells) were labelled for 20 min with 3 mCi ^{34}S-methionine before centrifugal elutriation. The isolated subpopulations were lysed and immunoprecipitated with anti-v-*myc* 12C as previously described except that the final washed immune complex was dissolved by boiling for 1 min in 9.5 M urea, 0.5% SDS, 5% β-mercaptoethanol, 0.2% ampholines *p*H 3.5–10 followed by addition of an equal volume of 9.5 M urea, 2% w/v NP40, 1.6% ampholines *p*H 5.0–7.0, 0.4% ampholines *p*H 3.5–10.0, 5% β-mercaptoethanol and boiling for 1 min. Equilibrium isoelectroc focusing was carried out as described by O'Farrell[25]. Briefly, the solubilized immune complex was applied to a first-dimension equilibrium isoelectric focusing tube gel. Following electrophoresis to equilibrium, the gel was removed, equilibrated in 10% glycerol, 5% β-mercaptoethanol, 2.3% SDS, 62.5 mM Tris-Cl *p*H 6.8, and electrophoresed in the orthogonal direction on 10% SDS-polyacrylamide slab gels. Only the relevant portions of the second-dimension gels are shown. The *p*H gradients in the isoelectric focusing gels were determined by incubating 5-mm slices of a parallel gel in degassed distilled water and measuring the *p*H of the eluted ampholine.

we measured c-*myc* protein synthesis in QEF released from the double-thymidine block into serum-free medium (Fig. 2*f*). In contrast to the cells released into complete medium, these cells showed only a small increase in incorporated ^{3}H-thymidine label at 4 h after release, no cell division up to 25 h after release and a significant decrease in ^{35}S-methionine incorporation. Figure 2*f* shows that these arrested quiescent cells had virtually the same levels of c-*myc* protein synthesis as the actively growing cells released into complete medium throughout most of the 25-h period (compare Fig. 2*e* and *f*). The transient changes in c-*myc* protein synthesis observed 1 h after release would seem to be a serum-dependent effect on synthesis rather than a cell cycle-regulated event.

To relate the levels of c-*myc* protein synthesis observed during the cell cycle of proliferating cells to the levels found in non-proliferating cells arrested in G_1, we sought to determine whether the levels of c-*myc* protein differed between quiescent G_1 cells deprived of serum and cells growing in the presence of serum. To obtain quiescent cells, we grew human K562 cells to saturation, and QEF to confluency, in serum-containing medium for 48 h followed by a further 48-h incubation in medium with 0.1% serum. The serum-starved K562 cells showed a 10-fold less ^{3}H-thymidine uptake than the parallel culture of proliferating cells. Both populations showed substantial c-*myc* protein syn-

thesis, although the proliferating cells had twice the level of c-*myc* protein (Fig. 3*a*). The QEF also showed a decrease in ^{3}H-thymidine incorporation. Cytofluorometric measurement of the DNA content of the serum-starved QEF showed a complete absence of S and G_2/M cells, in contrast to the non-serum-starved cells which showed the expected levels of S and G_2/M cells (Fig. 3*c*). Figure 3*b* shows that there is no significant difference in c-*myc* protein levels in proliferating and non-proliferating QEF.

Although the synthesis of c-*myc* proteins seems not to change significantly during the cell cycle, two other properties relating to c-*myc* proteins, turnover and post-translational modification, could potentially vary in a cell cycle-dependent manner. Pulse-chase experiments indicate an extremely short apparent half-life (20–30 min) for avian v-*myc* and human c-*myc* proteins[17,23]. We therefore used this technique on chicken MSB-1 cells separated by centrifugal elutriation. After a 15-min pulse and a 60-min chase, there was no significant change in apparent turnover of c-*myc* protein as a function of the cell cycle (data not shown).

To examine post-translational modification of c-*myc* protein as a function of the cell cycle, we performed two-dimensional gel electrophoretic analyses. The only known modification of *myc* proteins is phosphorylation[17,22,24]. Changes in the extent of phosphorylation, or some other type of charge modification, would probably produce charge alterations that would be detectable as shifts in isoelectric point[25]. MSB-1 cells labelled with ^{35}S-methionine for 20 min were separated by elutriation, lysed and the anti-v-*myc* 12C immunoprecipitate dissolved and subjected to equilibrium isoelectric focusing in the horizontal dimension and SDS-polyacrylamide gel electrophoresis in the vertical dimension[25]. Figure 4 shows that the avian p59^{myc} protein is resolved into a major spot of *p*I 6.0 which trails into a poorly resolved minor spot of *p*I 5.8. The minor p62^{myc} protein and another faint spot also have *p*Is of 6.0, suggesting that the difference between p59^{myc} and the higher-M_r forms is not due to hyperphosphorylation or other charge-altering modifications. Comparison of the two-dimensional electrophoretic patterns of the c-*myc* proteins in MSB-1 cells separated into different cell cycle subpopulations revealed no alterations in isoelectric point (Fig. 4). Finally, we have also failed to detect differences in ^{32}P-labelling of c-*myc* proteins at different times after release of MSB-1 cells from a G_1/S block (data not shown).

Our results demonstrate that in avian and human cells the rate of synthesis (as a fraction of total cell protein synthesis), turnover and modification of c-*myc* proteins is independent of the cell cycle stage. Several reasons make it unlikely that we did not achieve an effective separation of cells into different cell cycle stages. The increases observed in cell volume, nuclear volume and DNA content (Fig. 1*a*) and in protein synthesis and ribosomal RNA content in our isolated subpopulations all correlate well with progression through the cell cycle[11,12]. Furthermore, the accompanying manuscript shows that the levels of c-*myc* RNA in avian cells are also constant throughout the cell cycle, even though levels of histone 2b and thymidine kinase messenger RNAs vary during the cell cycle in the same cells[26]. Previous reports have indicated that stimulation of quiescent cells with growth factor results in a rapid increase in c-*myc* transcription[8]. One interpretation of these previous findings has been that c-*myc* is 'switched on' during the transition from a G_0 to a proliferating state and that high levels of c-*myc* expression may be needed to traverse each G_1 period[27,28]. However, the data in this and the accompanying paper demonstrate that the levels of c-*myc* expression are essentially the same in all growth phases of proliferating cell populations and in non-proliferating cells arrested in G_1 or G_1/S. We conclude that the characteristic mode of c-*myc* expression is continuous synthesis and rapid turnover throughout the cell cycle in non-terminally differentiated cells and tumour cells. The increase in c-*myc* expression on stimulation of resting cells is only transient, with the elevated levels of both c-*myc* RNA[8,10] and protein[29] falling back to a constant basal level which is then maintained throughout subsequent cell cycles.

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1. Royer-Pokora, B. *et al. Cell* **13**, 751–760 (1978).
2. Palmieri, S., Kahn, P. & Graf, T. *EMBO J.* **2**, 2385–2389 (1983).
3. Land, H., Parada, L. F. & Weinberg, R. A. *Science* **222**, 771–778 (1983).
4. Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenshein, G. E. *Cell* **36**, 241–247 (1984).
5. Gonda, T. J. & Metcalf, D. *Nature* **310**, 249–251 (1984).
6. Lachman, H. M. & Skoultchi, A. I. *Nature* **310**, 592–594 (1984).
7. Westin, E. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2490–2494 (1982).
8. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. *Cell* **35**, 603–610 (1983).
9. Makino, R., Hayashi, K. & Sugimura, T. *Nature* **310**, 697–698 (1984).
10. Greenberg, M. E. & Ziff, E. B. *Nature* **311**, 433–438 (1984).
11. Killander, D. & Zetterberg, A. *Expl Cell Res.* **38**, 272–284 (1965).
12. Zetterberg, A. *Expl Cell Res.* **42**, 500–513 (1966).
13. Alterman, R. B. M. *et al. Molec. cell. Biol.* **4**, 123–132 (1984).
14. Thompson, C. B. *et al. J. Immun.* **133**, 2333–2342 (1984).
15. Lozzio, C. B. & Lozzio, B. B. *Blood* **45**, 321–344 (1975).
16. Collins, S. J. & Groudine, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4813–4817 (1983).
17. Hann, S. R. & Eisenman, R. N. *Molec. cell. Biol.* **4**, 2486–2497 (1984).
18. Taub, R. *et al. Proc. natn. Acad. Sci. USA* **79**, 7837–7841 (1982).
19. Neel, B., Jhanwar, S., Chaganti, R. & Hayward, W. S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7842–7846 (1982).
20. Schubach, W. & Groudine, M. *Nature* **307**, 702–708 (1984).
21. Pachl, C., Schubach, W., Eisenman, R. & Linial, M. *Cell* **33**, 335–344 (1983).
22. Hann, S. R., Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. *Cell* **34**, 789–798 (1983).
23. Eisenman, R. *et al. Cold Spring Harb. Symp. quant. Biol.* **44**, 1215–1223 (1980).
24. Ramsay, G., Hayman, M. J. & Bister, K. *EMBO J.* **1**, 1111–1116 (1982).
25. O'Farrell, P. H. *J. biol. Chem.* **250**, 4007–4021 (1975).
26. Thompson, C. B., Challoner, P. B., Neiman, P. E. & Groudine, M. *Nature* **this issue**
27. Kelly, K., Cochran, B., Stiles, C. & Leder, P. *Curr. Topics Microbiol. Immun.* **113**, 117–126 (1984).
28. Pardee, A. B., Campisi, J., Gray, H. E., Dean, M. & Sonenshein, G. in *Mediators in Cell Growth and Differentiation* (eds Ford, R. J. & Maizel, A. L.) 21–29 (Raven, New York, 1985).
29. Persson, H., Henninghausen, L., Taub, R., DeGrado, W. & Leder, P. *Science* **225**, 687–693 (1984).

Isolation of a cDNA clone corresponding to an X-linked gene family (XLR) closely linked to the murine immunodeficiency disorder *xid*

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The striking number of human and murine immunodeficiency disorders which map to the X chromosome (Table 1) suggests that genes localized on this chromosome must have important roles in lymphocyte development. At least seven distinct disorders in the human and two in the mouse disrupt lymphocyte maturation, particularly that of B cells, at characteristic stages[1,2]. As functional genes mapping to the X chromosome in one mammal are found on the X chromosome in all other mammals[3], the same genes regulating lymphocyte development are expected to be found on the X chromosome in mouse and man. Investigations into the possible mechanisms of these X-linked disorders have been hampered by the lack of molecular probes for the genes or gene products affected; because of this, and the possibility of correlating

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one or more of the several hundred B- or T-cell-specific genes[4] with a specific mutation, we surveyed 15 different B- and T-cell-specific cDNA clones for localization to the X chromosome. We report here the characterization of one of these murine cDNA clones, which hybridizes with a large, X-linked gene family, designated XLR (X-linked, lymphocyte-regulated). We show that the XLR gene family is closely linked to the X-linked immunodeficiency described in the CBA/N mouse strain (*xid*)[5], by restriction fragment length polymorphism (RFLP) analysis of DNA from mice congeneic for *xid*. This finding, together with data on the expression of the XLR locus in B cells (see accompanying paper[6]), indicates that this gene family either includes the locus defined by the *xid* mutation or is adjacent to it in a gene complex which may be important in lymphocyte differentiation.

The initial cDNA clone, pX310, which identified XLR was obtained by screening a T-cell-specific, selected cDNA library[7] with a T-helper cDNA–T-suppressor mRNA-subtracted cDNA probe. Although pX310 was originally isolated in this manner as a T-cell-specific clone, subsequent analysis shows that mRNAs which cross-hybridize with this cDNA are also expressed in late-stage and secretory B-lymphocyte tumour lines, but not in the original B-cell tumour (BAL 17) used in the subtraction of the T-cell library (see accompanying report[6]). pX310 was one of 15 clones, obtained from T-cell-specific or B-cell-specific libraries, which were analysed by Southern blotting to mouse–hamster somatic cell hybrids[8]. This cDNA clone characteristically hybridizes with 10–14 bands on Southern analysis, consistent with its recognizing a gene family, and *Eco*RI restriction digests of DNAs from the somatic cell hybrid lines indicate that all of the restriction fragments which hybridize to pX310 are found on the mouse X chromosome (Fig. 1). Most useful for such a chromosomal assignment are results obtained from an analysis of the somatic cell hybrids MAE 28, containing the murine chromosomes 12 and X; MAE 32, containing chromosomes 16 and X; and MACH 4A63, containing most of the mouse chromosomes, including chromosomes 12 and 16 but not X (see Fig. 1). DNAs from the former two cell lines hybridize with pX310, while DNA from the latter line does not. None of 14 other cDNA clones obtained from the T- or B-cell-enriched cDNA libraries show evidence of being derived principally from X-chromosomal genes, or of recognizing such a large gene family. That the multiple bands seen with the pX310 probe are not due to 5′- or 3′-untranslated region repeats is indicated by the fact that an internal restriction fragment from an apparently full-length XLR cDNA clone gives an identical pattern on Southern blots (data not shown).

The 10–14 bands seen on Southern analysis of pX310 are comparable to the 15–28 bands seen with murine class I major histocompatibility complex (MHC) gene probes[9,10] (corresponding to ~35 different genes[11]) and to the 8–10 bands seen for α-interferon (IFN-α) corresponding to ~10 distinct genes[12].

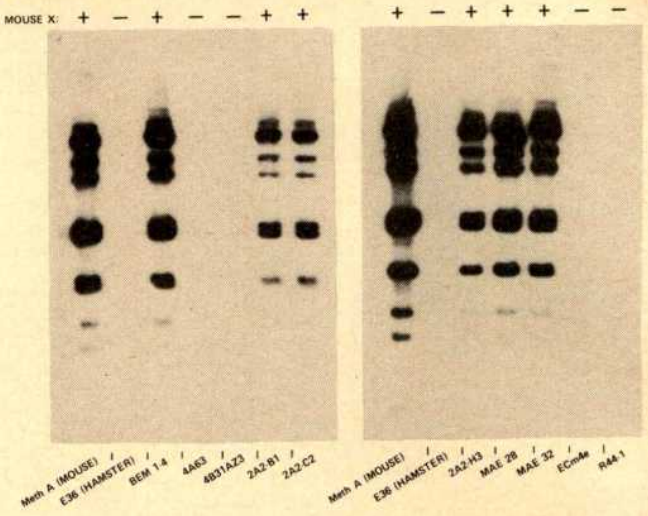


Fig. 1 X-linkage of the XLR gene family. DNAs were isolated from mouse–hamster somatic cell hybrids, digested with the restriction enzyme *Eco*RI, and blotted onto nitrocellulose according to the method of Southern[8]. The insert from XLR cDNA clone pM1 was labelled by nick-translation to 5×10^8 c.p.m. μg^{-1}, blots were hybridized with 10^6 c.p.m. ml^{-1} in 5× SSPE[28], 50% formamide at 42 °C, and washed repeatedly before exposure, the most stringent wash being in 0.2× SSPE at 60 °C. The names of hybrid lines are given at the bottom, and the presence (+) or absence (−) of the mouse X chromosome in each hybrid is indicated above each lane. The chromosomal content of each line has been described elsewhere[29,30], and the copies of mouse chromosome X per hybrid cell are: meth A (2.0), E36 (0), BEM 1-4 (0.87), 4A63 (0), 4B31AZ3 (0), 2A2-B1 (0.35), 2A2-C2 (0.60), 2A2-H3 (0.70), MAE 28 (1.00), MAE 32 (1.00), ECm4e (0), and R44-1 (0).

Similar to the class I MHC genes, there are no apparent dispersed members on other chromosomes. While an XLR probe gives little or no cross-hybridization with hamster DNA in moderately stringent conditions (see Fig. 1 legend), it does hybridize well with DNAs from three distinct wild mouse species and, indeed, shows less polymorphism than another gene family, PGK, for these DNAs (D.I.C. and M.M.D., unpublished data). This is distinctly different from the pattern seen for mobile elements[13]. Both class I MHC and IFN-α genes are genetically linked in a cluster to other very distantly related genes; in the former case, to the class II MHC genes[14], and in the latter case, to β-interferon[15].

One of the most studied X-chromosomal defects is the *xid* lesion[16,17], which causes a recessive B-cell immunodeficiency which appears to disrupt late-stage B-cell functions[18]. The mutation was recognized in a subline of mice, designated CBA/N,

Table 1 X-linked immunodeficiency diseases

Name of defect	Cell types affected	Presumed stage	Functional defect
Human defects (ref. 1)			
Severe combined immunodeficiency	T, B	Stem cell	T-cell immunity (TCI), antibody (Ab)
Wiskott–Aldrich syndrome	T, B, platelets	Haematopoietic	TCI, Ab to polysaccharides, low platelets
Agammaglobulinaemia (Bruton's)	B	Pre-B cell	Ab
Agammaglobulinaemia with growth hormone deficiency	B	Pre-B cell	Ab
Lymphoproliferative syndrome	B	B-cell growth regulation	Lymphomas on Epstein–Barr virus infection
Hyper IgM, reduced IgG and IgA	B	Plasma cells	Low IgG, low IgA
Hyper IgM and IgG, reduced IgA	B	Plasma cells	Low IgA
Murine defects (refs 2, 19, 20)			
CBA/N (*xid*)	B	Mature B cell	Antibody to polysaccharides
DBA/2Ha	B	Mature B cell	Plasma cell differentiation

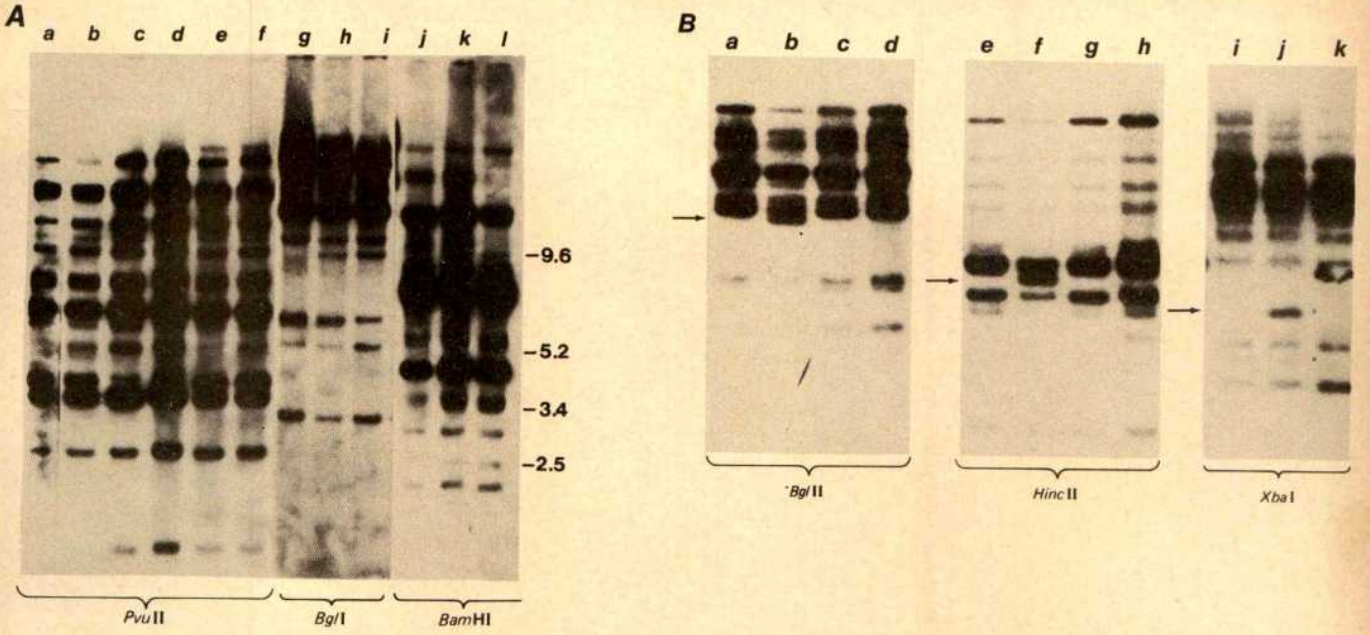


Fig. 2 *A*, Restriction fragment length polymorphism analysis of genomic DNA from MRL (lanes *a*, *g*), CBA/N (*c*, *i*, *l*), CBA/Ca (*d*), BXSB (*e*, *j*), and from the *xid* congeneic strains, MRL *xid* (*b*, *h*) and BXSB *xid* (*f*, *k*). Polymorphisms referred to in the text are labelled by size (in kb) on the right of the figure; restriction enzymes are indicated at the bottom. Blots were probed with the insert from the XLR cDNA clone, pX310. *B*, Southern comparison of genomic DNA from NZB (lanes *a*, *e*, *i*), NZB *xid* (*b*, *f*, *j*), CBA/N (*c*, *g*, *k*) and CBA/Ca (*d*, *h*) congeneic strains hybridized with XLR probe, pM1. Restriction enzymes were *Bgl*II (*a*–*d*), *Hinc*II (*e*–*h*), and *Xba*I (*i*–*k*). Bands unique to NZB *xid* are indicated by arrows. Using the formulation of Klein[14] and the fact that BXSB *xid*, MRL *xid* and NZB *xid* mice had been bred through 9, 12 and 19 potential recombinations, respectively, at the time of analysis, the probability of linkage to within 10 centimorgans is $1-(0.43)(0.31)=87\%$ for the two strains with classic RFLP, and to within 5 centimorgans, 85%, for all three strains.

derived from the normal strain, CBA/Ca. We compared the organization of the XLR locus in genomic DNA from the mutant CBA/N with that from the wild-type CBA/Ca strains, and genomic DNA from a mouse line bearing a distinct X-linked immunodeficiency, DBA/2Ha[19,20], with that from its normal parental line, DBA/2J. These comparisons revealed no obvious reorganizations or deletions in the XLR family in either the CBA/N (Fig. 2*B*, lanes *c*, *g* and *k*) or DBA/2Ha strains (data not shown). However, many eukaryotic mutations are not obvious deletions or translocations and are not directly detectable by Southern analysis[21–23]. It is conceivable that the complexity of the Southern pattern could obscure such a difference, although the patterns given by many different restriction enzymes have been analysed.

Congeneic strains have been bred in which the *xid* mutation, was introduced onto the background of the MRL strain (MRL *xid*[24]), the BXSB strain (BXSB *xid*[25]), and the NZB strain (NZB *xid*[26]); these congeneic lines are useful for determining whether a gene probe such as XLR, recognizes DNA linked to the *xid* mutation. The MRL, BXSB and NZB backgrounds are wild type for the *xid* phenotype. The congeneic mice are bred by backcrossing male offspring, which have been phenotyped for the *xid* trait, to background females, taking resulting F_1 (+/*xid*) females (the only circumstance in which recombination can occur) for crossing to background males, and typing their male offspring for use in continuing the line. This breeding strategy creates a line deriving almost all of its genetic material from the background strain, and only a small fraction of genetic material, almost exclusively surrounding the locus being introduced (*xid*), from the donor strain (see Fig. 2 legend for calculations)[14]. In all cases these mice are statistically expected to derive less than $\frac{1}{8}$ of their X chromosome from the *xid* donor[14]. If the XLR probe recognizes RFLPs derived from the *xid* donor, then some (or all) of the gene family must be linked to *xid*, as any unlinked DNA derived from the CBA/N background has essentially been bred out of the line.

Figure 2*A* shows the Southern blot analysis of male liver DNA prepared from the indicated murine strains, and used in RFLP analysis of the XLR gene family: both MRL *xid* (lane *b*) and BXSB *xid* (lane *f*) congeneic mice possess a 5.2-kilobase (kb) *Pvu*II band of the XLR family characteristic of CBA/N (*xid*) DNA (lane *c*) and unlike the banding pattern of the background strains, MRL (lane *a*) and BXSB (lane *e*). Furthermore, the MRL *xid* mice possess a 9.6-kb *Bgl*I polymorphism (lane *h*), not present in the MRL strain (lane *g*), that is characteristic of *xid* DNA (lane *i*). The BXSB *xid* strain (lane *k*) has inherited two *Bam*HI polymorphisms (at 3.4 and 2.5 kb) from the *xid* background (lane *l*) that are absent from the BXSB (lane *j*). In contrast, BXSB *xid* mice (lane *k*) also have the BXSB (lane *j*) form of a 15-kb *Bam*HI polymorphism, indicating that the BXSB *xid* line has inherited some of its XLR locus from the CBA/N DNAs, and some from the BXSB. If all members of the gene family are tightly clustered, like the MHC genes[11] and most gene families, recombination must have occurred between members of the XLR family. If the family is more dispersed, some members will be linked and more distant members unlinked. In either case, we conclude that one or more members of the XLR gene family are closely linked to *xid*.

Interestingly, in comparing multiple restriction digests from the NZB, NZB *xid* and CBA/N DNAs, rather than observing distinct RFLPs, we found bands unique to the NZB *xid* genome that were not present in either of the parental DNAs (Fig. 2*B*, lanes *b*, *f* and *j*). The 5.5-kb *Bgl*II, 3.6-kb *Hinc*II and 2.9-kb *Xba*I restriction fragments unique to the NZB *xid* strain are indicated by arrows (Fig. 2*B*). This observation suggests that during the breedng of the NZB *xid* line, meiotic rearrangement of the XLR locus occurred. The fact that this distinct pattern has been perpetuated for three subsequent generations, and not lost in backcrossing (D.I.C., A.D.S. and M.M.D., unpublished observation), also argues for linkage of XLR to the *xid* phenotype in this congeneic strain. Although these findings cannot be unambiguously interpreted, they may represent the result of an inversion or unequal recombination between distinct members of the gene family during the breeding of this strain. In the globin gene family, unequal recombinational events have been associated with gene deletions and reorganizations[27].

The multiple members of the XLR gene family may encode functionally distinct molecules and thus might be responsible for more than one of the X-linked deficiencies noted in Table

1. One such possibility is raised by the fact that the human X-linked Wiskott-Aldrich immunodeficiency causes a B-cell defect very similar to that of the *xid* mouse, but in addition leads to abnormal T cells and platelets[1]. Consistent with this possibility, XLR genes are expressed in both B and T lymphocytes (see accompanying report[6]). The isolation of the XLR probe described here defines a large number of new X-linked genes, at least some of which are linked to a murine immunodeficiency trait and expressed in lymphocytes. Further, it offers an important basis for the analysis of X-linked defects in the mouse and, when human probes become available, in the human equivalents.

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1. Waldmann, T. A., Strober, W. & Blaese, R. M. in *Clinical Immunology* Vol. 1, 314-375 (W. B. Saunders, Philadelphia, 1980).
2. Scher, I. *Adv. Immun.* **33**, 1-71 (1982).
3. Ohno, S. *A. Rev. Genet.* **3**, 495-524 (1969).
4. Davis, M. M. *et al. UCLA Symp.* **24**, 215-220 (1982).
5. Amsbaugh, D. F. *et al. J. exp. Med.* **136**, 931-949 (1972).
6. Cohen, D. I., Steinberg, A. D., Paul, W. E. & Davis, M. M. *Nature* **314**, 372-374 (1985).
7. Davis, M. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 2194-2198 (1984).
8. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
9. Shin, H. S., Stavnezer, J., Artzt, K. & Bennett, D. *Cell* **29**, 969-976 (1982).
10. Silver, L. M. *Cell* **29**, 961-968 (1982).
11. Steinmetz, M., Winoto, A., Minard, K. & Hood, L. *Cell* **28**, 489-498 (1982).
12. Goeddel, D. V. *et al. Nature* **290**, 20-26 (1981).
13. Steffen, D. & Weinberg, R. A. *Cell* **15**, 1003-1010 (1978).
14. Klein, J. in *Biology of the Mouse Histocompatibility Complex*, 16-39 (Springer, New York, 1975).
15. Owerbach, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 3123-3127 (1981).
16. Paul, W. E. *et al.* in *Cells of Immunoglobulin Synthesis*, 383-396 (Academic, New York, 1979).
17. Mosier, D. E. *et al. Immun. Rev.* **37**, 89-104 (1977).
18. Ahmed, A. *et al. J. exp. Med.* **145**, 101-110 (1977).
19. Takatsu, K., Tominaga, A. & Hamaoka, T. *J. Immun.* **124**, 2414-2422 (1980).
20. Takatsu, K. *et al. Nature* **292**, 360-362 (1981).
21. Orkin, J. H. *et al. Nature* **296**, 627-631 (1982).
22. Giannelli, F. *et al. Nature* **303**, 181-182 (1983).
23. Wiginton, D. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 7481-7485 (1983).
24. Steinberg, E. B. *et al. J. Immun.* **131**, 2789-2795 (1983).
25. Smith, H. R., Chused, T. M. & Steinberg, A. D. *J. Immun.* **131**, 1257-1262 (1983).
26. Steinberg, A. D. *et al. Immun. Rev.* **55**, 121-154 (1981).
27. Jones, R. W., Old, J. M., Trent, R. J. & Weatherall, D. J. *Nature* **291**, 39-44 (1981).
28. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning*, 447 (Cold Spring Harbor Laboratory, New York, 1982).
29. D'Eustachio, P., Bothwell, A. L. M., Tokaro, T. K., Baltimore, D. & Ruddle, F. H. *J. exp. Med.* **153**, 793-800 (1981).
30. Goff, S., D'Eustachio, P., Ruddle, F. H. & Baltimore, D. *Science* **218**, 1317-1319 (1982).

Expression of an X-linked gene family (XLR) in late-stage B cells and its alteration by the *xid* mutation

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One of the most extensively studied X-linked immunodeficiency disorder is the *xid* mutation of the mouse strain CBA/N (reviewed in ref. 1). This mutation may involve a maturational defect as *xid* animals are unable to raise antibodies to soluble polysaccharide antigens, a function normally attributed to late-stage B cells[2,3]. Moreover, studies using monoclonal antibodies have defined a B-cell surface antigen (BLA-2 or 14G8) that is expressed on most or all immature B lymphocytes, but not on a subpopulation of mature splenic B lymphocytes[4,5]; this late-stage, 14G8 antigen-negative splenic B-cell subpopulation is apparently absent from mice bearing the *xid* defect[5]. In the accompanying paper[6] we describe the isolation of a cDNA clone recognizing a family of genes on the X chromosome, at least some of whose members are closely linked to the *xid* trait. We report here that this gene family, XLR, is transcribed in certain B- and T-cell lineage tumours, but not in macrophage tumours, or liver or kidney cells. We show that it is transcribed principally in late-stage, 14G8-negative B-cell tumours and plasmacytomas, but not in immature B-cell or pre-B-cell tumours. We are able to detect transcription in all of 12 plasmacytomas (secretory B-cell tumours) derived from mice with normal X chromosomes, but not in three plasmacytomas carrying the *xid* mutation. These data, combined with the restriction fragment length polymorphism analysis linking the XLR gene family to the *xid* mutation[6], suggests that the *xid* defect occurs within a member of this gene family.

We analysed RNA expression from the XLR gene family in several B-cell tumours and other tissues, by Northern analysis and, in some cases, primer extension studies. Expression of XLR occurs in only certain B-cell tumours (Figs 1, 2); it is also transcribed in a wide variety of functional T-cell hybridomas,

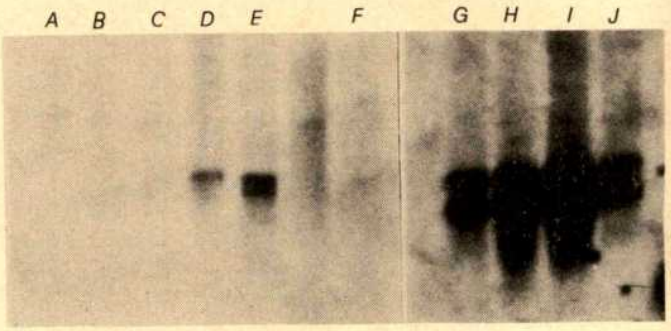


Fig. 1 Northern analysis of 5 μg poly(A)$^+$ RNA electrophoresed through 5 mm MeHgOH and 1.5% agarose, as described elsewhere[30]. Lanes *A–F* were probed with pX310, a cDNA clone recognizing the XLR gene family; lanes *G–J* were probed with a β_2-microglobulin cDNA cones[31]. The RNA samples were from kidney (*A*, *G*), liver (*B*, *H*), BCL1 (*C*, *I*), M315P (non-secretory surface IgA$^+$ lymphoma, *D*), M315J (secretory IgA plasmacytoma, *E*, and 2043-1 (*F*, *J*). In all cases blots were probed with an XLR clone, then with a β_2-microglobulin clone to ensure that each RNA was intact and hybridizable.

of both helper and suppressor phenotype, and in other T-cell tumours. In contrast, liver cells, kidney cells (Fig. 1*A*, *B*) and the macrophage tumour lines J774 (ref. 7) and P338D1 (ref. 8) (data not shown) produced no detectable XLR transcripts. We detected transcription in normal lymphoid tissue by Northern analysis of splenocytes (Fig. 2*E*) and thymocytes, or by primer extension studies of purified B cells[9] (data not shown). We conclude that XLR is expressed in some B- and T-cell lymphomas, and in normal lymphocytes, but not in several kinds of non-lymphoid cells; the specificity of this expression during B-cell ontogeny is discussed below.

The level of transcription of XLR genes is ~5–10 copies per cell for XLR-expressing B-cell tumours. This measurement reflects both the frequency of positively hybridizing clones from a plasmacytoma cDNA library (1 clone per 50,000 colonies) and the ratio of XLR to μ (heavy chain) RNA in the presecretory B lymphoma L10A, which is 1/50 as assayed by cytoplasmic RNA titration[10]. This level of expression makes it rare[11], and it is one of several rare lymphocyte-specific cDNA clones which we have isolated using subtracted cDNA probes[12,13]. Interestingly, XLR expression seems to be even lower in normal cell populations (Fig. 2, compare lanes *B–D* with *E*)[14]. Scanning



Fig. 2 Northern analysis of total RNA electrophoresed through 1M formaldehyde, 1.5% agarose and blotted onto nitrocellulose, as described elsewhere[32]. The probe is pM1, a full-length XLR cDNA clone. RNAs in lane *A* (10 μg) and *B*–*D* (20 μg) were from NZB plasmacytomas (2154 (*A*), 2454 (*B*), 2880 (*C*) and 2997 (*D*) from M. Weigart); *E*, whole NZB splenocyte RNA (25 μg). The results are from the same blot and probe but lane *E* (21-day exposure) was exposed seven times longer than lanes *A*–*D* (3-day exposure).

densitometry establishes that the most weakly hybridizing NZB plasmacytoma produces a signal 25-fold more intense than NZB splenocytes in equivalent assay conditions; this relationship remains unchanged when polyadenylated RNAs are compared (data not shown).

As XLR expression is observed in only some B-cell tumours, we attempted to determine whether the types of B-cell tumours expressing XLR RNA represent a specific stage of B-cell differentiation. We addressed this question by analysing an extensive panel of B-cell tumours (Table 1), because such tumours have been an effective model for normal B-cell maturation in many systems[15,16]. The earliest identifiable normal B-cell precursor is the pre-B cell[17]. As shown in Table 1, only one of nine Abelson pre-B-cell tumours[16] expresses XLR. Immature B cells, such as those found in neonatal or *xid* mutant mice, possess large amounts of surface IgM[18] and typically express the 14G8 determinant[4,5]; neither of the two early-stage B-cell tumours assayed here transcribes XLR. More mature B cells express δ (heavy chain)[19] and many do not express 14G8 (refs 5, 7). Furthermore, as a requirement for immunoglobulin secretion, presecretory B cells must activate the gene encoding the J-chain protein because this protein is necessary for assembling the secreted, multimeric forms of IgM and IgA[20]; therefore, activation of the J-chain gene provides an independent means of assessing B-cell maturity[21]. Of six B-cell lymphomas analysed which possess a mature surface immunoglobulin phenotype, three are relatively more mature in that they express little or no 14G8, and in that they have activated the J-chain gene as demonstrated by either demethylation (L10A)[21] or actual transcription (Fig. 3*C*, lanes *e* and *g*). Of this group of six lymphomas, only the three more mature, presecretory B-cell-stage tumours transcribe XLR. The final stage of B-cell differentiation is immunoglobin secretion and all of 12 secretory-stage tumours (plasmacytomas) transcribe XLR. The pattern of XLR transcription differs between plasmacytomas, which produce two principal transcripts of 1.0 and 1.1 kilobases (kb), and presecretory tumours, which produce one distinguishable transcript of 1.2 kb (Fig. 1*D*, *E*). This finding indicates that either multiple members of the family are activated sequentially or differential splicing occurs during the transition from the presecretory to the secretory stage, as occurs with the μ heavy-chain gene[22]. Even though these stages are probably not absolute, B-cell tumours which transcribe XLR are mature cells at either the presecretory or secretory stage when analysed by multiple criteria (Table 1).

It has been established that a mature subpopulation of splenic B lymphocytes lacking the 14G8 (BLA-2) antigen, and uniquely characterized by a panel of other markers, is absent from mice with the *xid* mutation[5]. Interestingly, the B-cell tumours analysed in the present report, which transcribe XLR, also lack the 14G8 marker. On the basis of this similarity, it is tempting to equate these B-cell tumours expressing XLR with the normal B cells which are absent from *xid* mutant mice. The 'turning-on' of this X-linked gene during the maturation of some B cells coincides with the 'turning-off' of a marker (14G8) which is expressed at an abnormally high frequency in *xid* B cells.

We next asked whether the *xid* mutation directly affects XLR expression. We prepared and analysed mRNA from a pool of three plasmacytomas raised in the NZB *xid* mouse[23], which is an NZB congeneic strain into which the *xid* mutation has been introduced[24], and compared the results with those obtained from plasmacytomas lacking the *xid* mutation, including NZB plasmacytomas. The 12 plasmacytomas lacking the *xid* mutation included cells using the μ, γ or α immunoglobulin heavy chains and κ or λ light chains, and represented tumours expanded either *in vivo* or *in vitro*. All of these 12 plasmacytomas gave essentially similar patterns and amounts of XLR transcription whereas the pool of NZB *xid* plasmacytomas showed no detectable XLR expression (Fig. 3*A*, lane *h*). The most relevant comparison is with the four NZB lines (Fig. 2*A*–*D*). Allowing for the slightly greater amount of RNA loaded for the NZB *xid* pool, and the sensitivity of these Northern blots (see above), we estimate that XLR transcription must be decreased at least 30-fold in the NZB *xid* plasmacytomas, to less than 1 copy per cell; comparative titrations of plasmacytoma RNAs in primer extension experiments support this conclusion (data not shown). Figure 3*C*, lane *j* shows that the same NZB *xid* plasmacytoma RNA contains readily detectable J-chain mRNA, establishing

Table 1 XLR expression in B-cell tumours

Stage	XLR (no. positive/ no. tested)	Phenotype: Immunoglobulin	14G8 staining	J chain	Tumour (XLR expression + or −)
(1) Pre-B	1/9	Cytoplasmic μ	+	−	2043-1, 38B7, ABL5 1, 8, 19, 109, 122, 140 [all (−)] ABLS 5 (+)
(2) Early B	0/2	Surface IgM	+	−	BCL1, W231
(3) Intermediate B	0/3	Surface IgM, IgD	+	−	K46, X16C, BAL17
(4) Presecretory B	3/3	Surface immunoglobulin IgD	−	DM or +	L10A*, 315P, 2PK3
(5) Plasmacytoma	12/12	Secretory immunoglobulin	−	+	V17C, HM14, CPC1, TEPC 1197, CBB22PC3, CBPC112, N2BPC 2154, 2454, 2880, 2993, M315J, M104e

DM, demethylated, not expressed[21]. Data were taken from refs 15, 16, 21, 26–29 and the present study.
We tested all tumours in categories (2), (3) and (4) for 14G8 staining and J expression, but only representative tumours from categories (1) and (5).
* Ref. 21.

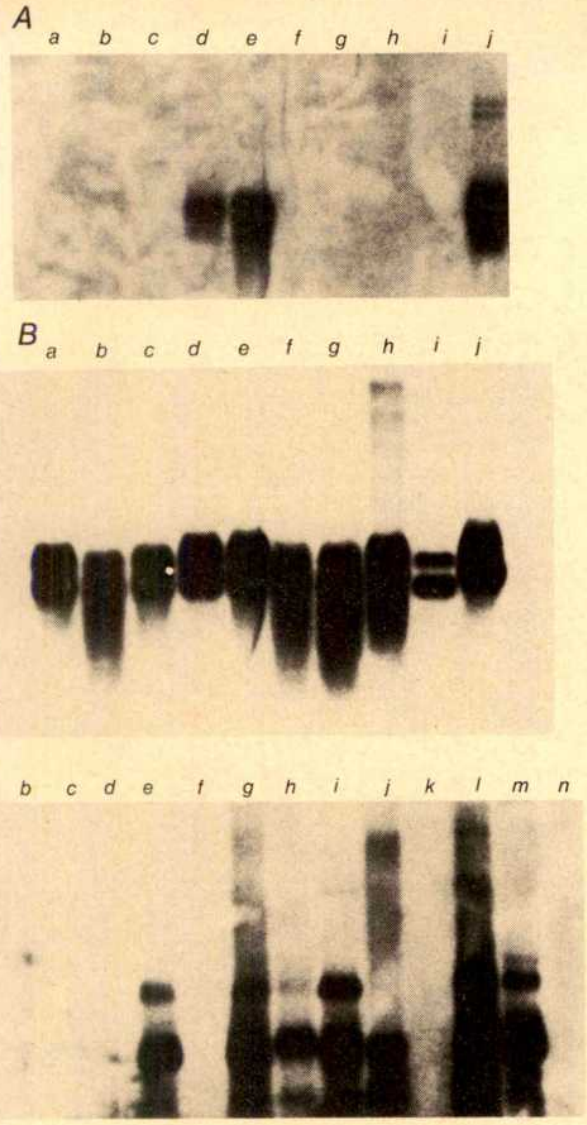


Fig. 3 Northern analysis, as in Fig. 2, using 30 μg of total NZB *xid* plasmacytoma, or 5 μg of poly(A)$^+$ RNA from the other tumours. *A*, Probed with pM1; *B*, the same blot probed with β_2-microglobulin. The RNAs in each lane are: *a*, WEH1 231 (W231); *b*, W231 (different batch); *c*, lipopolysaccharide (LPS)-stimulated W231; *d*, 2PK3; *e*, L10A; *f*, X16C; *g*, K46; *h*, NZB *xid* plasmactyoma; *i*, BAL 17; and *j*, M104e. *C*, RNA probed with a J-chain cDNA clone[15]; *a*, W231; *b*, BAL17; *c*, X16C; *d*, K46; *e*, 2PK3; *f*, L10A; *g*, M315P; *h*, M104e; *i*, M315J; *j*, NZB *xid* plasmacytoma; *k*, T-cell RNA from EL4; *l*, V17C; *m*, HM14; and *n*, LPS-stimulated W231. The NZB *xid* plasmacytoma RNA was extracted from a pool of equal numbers of cells from three independently derived plasmacytomas, in order to have sufficient material for complete analysis. One principal type of antibody produced by these cells is IgA/κ (A.D.S., unpublished data).

that these cells are at the correct stage to transcribe XLR (Table 1), and that the mRNA analysed is intact. We conclude that the *xid* mutation alters the normal expression of XLR.

The experiments described here establish that XLR transcription is characteristic of late-stage B-lymphocyte maturation and that this expression is altered in cells bearing the *xid* mutation. These data indicate that the *xid* mutation disrupts XLR gene expression and, coupled with the linkage data described in the accompanying paper[6], support the notion that the *xid* mutation occurs within a member of the XLR gene family. The mutation may act either directly or indirectly to account for the pattern of expression observed here. In the direct model, the NZB *xid* plasmacytoma cells would normally express XLR but cannot, owing to the *xid* defect. Alternatively, these plasma cells may represent an XLR-nonexpressing class of B cells that are rare in normal animals but common in those carrying the *xid* defect; this rare class would predominate if the XLR-requiring plasma cell fails to mature in the *xid* animals. The latter alternative is more consistent with a dual-pathway model of (late-stage) B-cell maturation[1,5,25].

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1. Paul, W. E. *et al.* in *Cells of Immunoglobulin Synthesis*, 383–396 (Academic, New York, 1979).
2. Ahmed, A. *et al. J. exp. Med.* **145**, 101–110 (1977).
3. Mosier, D. E. *et al. Immun. Rev.* **37**, 89–104 (1977).
4. Kung, J. T. *et al. J. Immun.* **128**, 2049–2056 (1982).
5. Hardy, R. R., Hayakawa, K., Parks, D. R., Herzenberg, L. A. & Herzenberg, L. A. *J. exp. Med.* **159**, 1169–1188 (1984).
6. Cohen, D. I. *et al. Nature* **314**, 369–372 (1985).
7. Lachman, L. B., Hacker, M. P., Blyden, G. T. & Handschumacher, R. E. *Cell. Immun.* **34**, 416–418 (1977).
8. Mizel, S. B., Rosenstreich, D. L. & Oppenheim, J. J. *Cell. Immun.* **40**, 230–241 (1978).
9. Howard, M. *et al. J. exp. Med.* **155**, 914–923 (1982).
10. Kemp, D. J., Harris, A. W., Cory, S. & Adams, J. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2876–2880 (1980).
11. Lewin, B. in *Gene Expression* Vol. 2, 2nd edn, 713–719 (Wiley, New York, 1980).
12. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308**, 149–153 (1984).
13. Davis, M. M., Chien, Y. H., Gascoigne, N. R. J. & Hedrick, S. M. *Immun. Rev.* **81**, 235–258 (1984).
14. Payne, G. S., Bishop, J. M. & Varmus, H. E. *Nature* **295**, 209–217 (1982).
15. Mather, E. L., Alt, F. W., Bothwell, A. L. M., Baltimore, D. & Koshland, M. E. *Cell* **23**, 369–389 (1981).
16. Siden, E. J., Baltimore, D., Clark, D. & Rosenberg, N. *Cell* **16**, 389–396 (1979).
17. Levitt, D. & Cooper, M. D. *Cell* **19**, 617–625 (1980).
18. Scher, I., Sharrow, S. O., Wistar, R. Jr, Asofsky, R. & Paul, W. E. *J. exp. Med.* **144**, 494–506 (1976).
19. Kearney, J. F. *et al. J. exp. Med.* **146**, 297–301 (1977).
20. Koshland, M. E. *Adv. Immun.* **20**, 41–69 (1975).
21. Yagi, M. & Koshland, M. E. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4907–4911 (1981).
22. Rogers, J. *et al. Cell* **20**, 303–312 (1980).
23. Steinberg, B. J. *et al. J. clin. Invest.* **70**, 587–597 (1982).
24. Klein, J. in *Biology of the Mouse Histocompatibility Complex*, 16–39 (Springer, New York, 1975).
25. Perlmutter, R. M. *et al. J. exp. Med.* **149**, 993–998 (1979).
26. Kim, K. J. *et al. J. Immun.* **122**, 549–554 (1979).
27. Lanier, L. L., Warner, N. L., Ledbetter, J. A. & Herzenberg, L. A. *J. Immun.* **127**, 1691–1697 (1981).
28. Slavin, S. & Strober, S. *Nature* **272**, 624–626 (1978).
29. Gebel, H. M., Autry, J. R., Rohrer, J. W. & Lynch, R. G. *J. natn. Cancer Inst.* **62**, 201–212 (1979).
30. Alwine, J. C., Kemp, D. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5350–5354 (1977).
31. Parnes, J. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 2253–2257 (1981).
32. Seed, B. in *Genetic Engineering* Vol. 4 (eds Setlow, J. K. & Hollaender, A.) 91–102 (Academic, New York, 1982).

An N-terminal peptide from p60^{src} can direct myristylation and plasma membrane localization when fused to heterologous proteins

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The *src* gene product, p60^{src}, of Rous sarcoma virus (RSV) is a tyrosine-specific protein kinase which is associated with the plasma membrane of infected cells (for review, see ref. 1). Myristic acid is bound in an amide linkage to glycine 2 of p60^{src} (refs 2–6). Of the N-terminal 30 kilodaltons of p60^{src}, only amino acids 1–14 are required for myristylation, and myristylation of p60^{src} may be required for its membrane association, and for cell transformation[4,7]. To test the hypothesis that the first 14 amino acids of p60^{src} contain a recognition sequence for myristylation, we have fused the DNA sequence coding for these amino acids to either the *fps*

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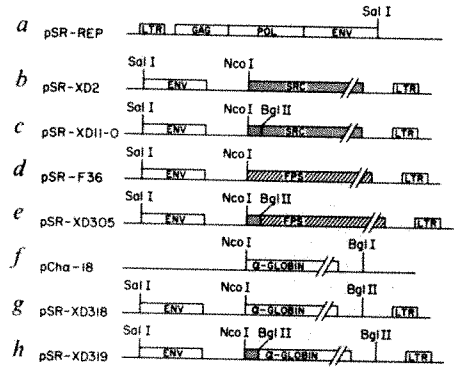


Fig. 1 Structures of the coding sequences of the plasmids used. The shaded boxes indicate portions of the *src* gene; hatched boxes, portions of the *fps* gene; open boxes, chimpanzee α-globin gene. All methods for recombinant DNA manipulations have been described previously[4,26]. All constructions were verified by restriction mapping, and by DNA sequence analysis in the case of pSR-XD305 and pSR-XD319. *a*, pSR-REP contains most of the genes required for replication of RSV (*gag*, *pol* and *env*), and was used in the transfection protocol to produce infectious virus containing the replacements for the *src* gene shown in *b*–*g*, as described elsewhere[26]. *b*, pSR-XD2 contains the wild-type RSV *src* gene[26]. *c*, pSR-XD11-0 is a pSR-XD2 derivative with a *Bgl*II linker inserted into the DNA coding for arginine 15 of p60^{src} (ref. 26). pSR-XD11-4 has a similar structure, except that two more base pairs are inserted into the DNA at the 5′ side of the linker[26]. *d*, pSR-F36 is similar to pSR-XD2, except that the *src* gene is replaced by a transforming fragment of the FSV *fps* gene. The construction and characterization of this clone have been described[9]. *e*, pSR-XD305 encodes a fusion protein between amino acids 1–14 of p60^{src} and amino acids 2–833 of the F36 transforming protein. This clone was constructed by first partially digesting pSR-F36 with *Nco*I, and with S_1 nuclease, followed by *Bgl*II linker (CAGATCTG) insertion. One clone, pF23, had a linker inserted at the remnant of the *Nco*I site which spans the initiation codon of the transforming protein of F36 (ref. 9), with S_1 digestion of only the protruding 5′ end remaining after *Nco*I digestion. This clone was recombined, at its *Bgl*II site, with pSR-XD11-0. *f*, pCh α-18 is a complete cDNA clone of a chimpanzee α-globin gene[10]. *g*, pSR-XD318, a derivative of pSR-XD2 with the pCh α-18 coding sequence substituted for *src*. pCh α-18 was cut with *Bgl*I (which cuts 3′ to the termination codon) followed by S_1 digestion and *Bgl*II linker insertion. This DNA was digested with *Bgl*II and *Nco*I. *Nco*I cuts at the initiation codon for α-globin. pSR-XDR111, a pSR-XD2 derivative with a *Bgl*II linker inserted at the *Rsa*I site 172 nucleotides 3′ to the termination codon of *src* (F.R.C. unpublished data), was digested with *Bgl*II and partially with *Nco*I. The fragment that was digested with *Nco*I only at the *src* initiation codon was ligated to the globin DNA insert to produce pSR-XD318. *h*, pSR-XD319 is a derivative of pSR-XD2 which encodes a fusion between the first 14 amino acids of p60^{src} and all of α-globin. pChα-18 was cut with *Bgl*I and S_1, then with *Nco*I. The *Nco*I site was gap-filled, and *Bgl*II linkers ligated to this insert DNA. pSR-XD321, a pSR-XD2 derivative in which all of *src* except for DNA encoding amino acids 1–14 is deleted (J. Samarut and F.R.C., unpublished), was constructed by recombining pSR-XD11-4 (see description of pSR-XD11-0 above) with pSR-XDR23, a pSR-XD2 derivative with a *Bgl*II linker inserted at an *Rsa*I site 105 nucleotides 3′ to the *src* termination codon (F.R.C., unpublished). After cutting of pSR-XD321 with *Bgl*II, followed by digestion with alkaline phosphatase, the *Bgl*II-ended α-globin insert was ligated into the plasmid.

gene of the F36 derivative of Fujinami sarcoma virus (FSV)[8,9], or to the chimpanzee α-globin gene[10]. We report here that although the fusion proteins were myristylated, the parental proteins were not, and unlike the non-myristylated F36 p91^{fps} which was not bound to the plasma membrane, the myristylated fusion protein was bound, like p60^{src}. We conclude that the first 14 amino acids of p60^{src} contain a sequence which is sufficient for myristylation, and which may direct proteins to the plasma membrane.

The structures of the genes inserted into the RSV genome instead of the *src* gene are shown in Fig. 1. Figure 2 shows the sequences of regions of interest.

```
                                    5                  10                  15
pSR-XD2 (src)          MetGlySerSerLysSerLysProLysAspProSerGlnArgArg...
                  ...CCATGGGGAGTAGCAAGAGCAAGCCTAAGGACCCCAGCCAGCGCCGG...
                     ------
                     NcoI

                                    5                  10                  15
pSR-F36 (fps)          MetGlyPheGlyProGluLeuTrpCysProLysGlyHisThrGlu...
                  ...CCATGGGCTTTGGGCCGGAGCTGTGGTGCCCGAAGGGGCACACTGAG...
                     ------
                     NcoI

                                    5                  10                  15
pSR-XD318 (globin)     MetValLeuSerProAlaAspLysThrAsnValLysAlaAlaTrp...
                  ...CCATGGTGCTGTCTCCTGCCGACAAGACCAACGTCAAGGCCGCCTGG...
                     ------
                     NcoI

                          10 (src)          LINKER     2 (fps)
pSR-XD305 (src-fps)  ....AspProSerGlnArg [ProAspLeu] GlyPheGlyProGlu...
                     ....GACCCCAGCCAGCGCC [CAGATCTG] GGCTTTGGGCCGGAG...
                                  ------
                                  BglII

                          10 (src)          LINKER     1 (globin)
pSR-XD319 (src-globin) ....AspProSerGlnArg [ProGlnIleTrp] MetValLeuSer...
                       ....GACCCCAGCCAGCGCC [CACAGATCTG] CATGGTGTCTCCT...
                                    ------
                                    BglII
```

Fig. 2 Sequences of the N-terminal portions of the coding sequences in pSR-XD2, pSR-F36, pSR-XD318, pSR-XD305 and pSR-XD319. The pSR-XD2, pSR-F36 and pSR-XD318 sequences are from refs 27, 8 and 10, respectively. DNA derived from *Bgl*II linkers and amino acids encoded by this DNA are shown in brackets. The *src* DNA in pSR-F36 is derived from pSR-XD11-0, and that in pSR-XD319 from pSR-XD11-4 (ref. 26).

The fragment of the *fps* gene expressed following transfection with the pSR-F36 plasmid (nucleotides 1,472–4,162 of the FSV *fps* gene) lacks the *gag* portion, and encodes an 833-amino acid *fps* protein (amino acids 349–1,182 of the FSV *fps* protein), called p91^{fps}. The plasmid pSR-XD305 (Figs 1, 2) contains a recombinant *src*–*fps* gene which encodes a protein consisting of the first 14 amino acids of p60^{src}, followed by Pro-Asp-Leu, then amino acids 2–833 of the F36 *fps* protein[9]. The viruses recovered following transfection with these plasmids are called F36 (ref. 9) and NY305, respectively.

A protein of relative molecular mass (M_r) 93,000 (93K) is specifically immunoprecipitated from extracts of NY305-infected cells by anti-*fps* antiserum (Fig. 3*A*). Proteolytic mapping (data not shown) confirms that this protein is the product of the NY305 *src*–*fps* fusion gene, p93$^{src-fps}$. The F36 p91^{fps} is not detectably labelled with ^{3}H-myristic acid, even after long exposure, whereas both p60^{src} and p93$^{src-fps}$ label well (Fig. 3*A*).

We have compared the subcellular localization of p91^{fps} and p93$^{src-fps}$ by cell fractionation and measurement of immune complex tyrosine kinase activity, as described previously[4,9]. As with the FSV transforming protein p140$^{gag-fps}$ (refs 11–13), the proportion of p91^{fps} found in the crude membrane pellet (P100) was salt-dependent (Fig. 4*A*). In contrast, 95% of the p93$^{src-fps}$ was found in the crude membrane pellet at both salt concentrations tested. The P100 fractions were floated in discontinuous sucrose gradients. Unlike p60^{src}, which is tightly and specifically associated with the plasma membrane[1], the p91^{fps} kinase activity was not enriched in any membrane fraction. The p93$^{src-fps}$ kinase activity was highly enriched in plasma membranes (Fig. 4*B*). Therefore, the addition of the first 14 amino acids of p60^{src} to p91^{fps} made the subcellular fractionation pattern of the fusion protein indistinguishable from that of p60^{src} (refs 1, 4, 14).

Both F36 and NY305 induce focus formation efficiently, and NY305 induces the same distinctive fusiform morphology as reported for F36, in contrast to the round cell morphology induced by RSV[9] (data not shown). As the RSV p60^{src} and NY305 p93$^{src-fps}$ proteins appear to have a similar subcellular location, transformed cell morphology may reflect the affinity of these enzymes for substrates, rather than the subcellular location of the tyrosine kinase. Like F36, NY305 induces colony formation in soft agar, and both viruses are tumorigenic (data not shown). Thus, the alteration in subcellular location of p91^{fps} by the addition of the first 14 amino acids of p60^{src} did not alter

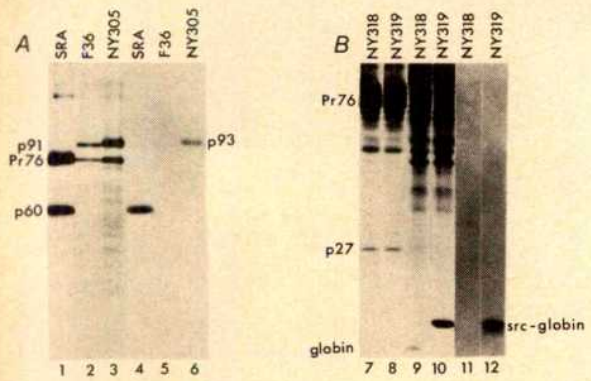


Fig. 3 Myristylation of fusion proteins containing the N-terminal 14 amino acids of p60^{src}. Infected chick embryo fibroblasts (CEF) were labelled for 4 h with ^{3}H-leucine or ^{3}H-myristic acid, and cell lysates were immunoprecipitated with saturating amounts of antiserum as described previously[4]. *A*, Infected cells were labelled with ^{3}H-leucine (lanes 1-3) or ^{3}H-myristic acid (lanes 4–6), and immunoprecipitated proteins were separated by electrophoresis on a 9% SDS-polyacrylamide gel. Proteins from cells infected with the Schmidt–Ruppin subgroup A strain of RSV (SRA; lanes 1, 4) were immunoprecipitated by tumour-bearing rabbit (TBR) serum[4]. Proteins from F36- (lanes 2, 5) or NY305-infected cells (lanes 3, 6) were immunoprecipitated by FSV-specific regressing-tumour antiserum (anti-FST; refs 9, 28). Exposure of the fluorographed gel for 2 days yielded lanes 1–3; exposure for 12 days lanes 4–6. *B*, Infected cells were labelled with ^{3}H-leucine for 0.5 h (lanes 7–10) or with ^{3}H-myristic acid for 2 h (lanes 11, 12), and immunoprecipitated proteins were analysed by electrophoresis on a 12.5% SDS-polyacrylamide gel. Proteins were immunoprecipitated with TBR serum (lanes 7,8) or with rabbit anti-human haemoglobin serum (Cappel Laboratories) (lanes 9–12). The fluorographed gel was exposed for 12 (lanes 7–10) or 16 (lanes 11, 12) days.

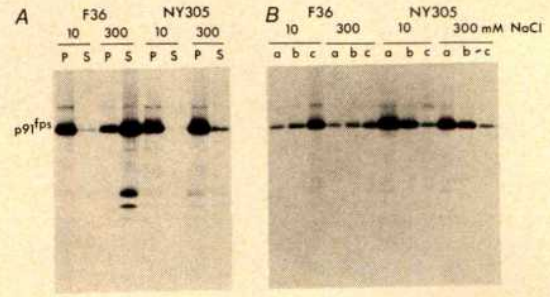


Fig. 4 Salt-independent plasma membrane association of a fusion protein containing the N-terminal 14 amino acids of p60^{src}. *A*, Infected cells labelled for 4 h with ^{3}H-leucine were fractionated into crude membrane (P) and cytosolic (S) fractions as described previously[4], except that the salt concentration of the postnuclear supernatant was adjusted to 10 mM or 300 mM as indicated. Kinase activity was assayed in immune complexes formed by immunoprecipitation with anti-FST serum as described previously[9,28]. Immunoprecipitates were analysed on a 9% polyacrylamide gel. Autophosphorylated bands were excised from the gel shown in Fig. 3*A*, and ^{32}P radioactivity quantitated by Cerenkov counting. The percentage radioactivity found in the P100, assuming 100% recovery, was as follows: for F36 p91^{fps}, 94% and 15% at 10 and 300 mM NaCl, respectively; for NY305 p93$^{src-fps}$, 96% and 93%, respectively. *B*, Crude membrane fractions prepared in *A* were fractionated on a discontinuous sucrose gradient as described previously[4], except that the gradient contained fewer steps: the P100 fraction was resuspended in 40% sucrose, underlaid with 50% sucrose, and overlaid with 35% and 20% sucrose in buffer. Lanes *a–c* represent membrane fractions collected from the gradient interfaces: *a*, 20/35% sucrose interface, which is enriched for plasma membranes; *b*, 35/40% sucrose interface; *c*, 40/50% sucrose interface, which is enriched for rough endoplasmic reticulum, Golgi and mitochondrial membranes[14]. Kinase activity was assayed in immune complexes formed by immunoprecipitation with anti-FST serum and analysed by electrophoresis on a 9% SDS-polyacrylamide gel. The radioactivity in the autophosphorylated bands was quantitated, and the specific activity of kinase activity in each fraction quantitated with reference to the total protein content, as described elsewhere[4]. p91^{fps} distributed essentially in parallel with the total protein of the fractions. p93$^{src-fps}$ showed an approximately 100-fold enrichment in the 20/35% fraction relative to that in the 40/50% fraction.

biological activity; this is in contrast to the requirement for myristylation and membrane association of p60^{src} for cell transformation[4,7], and demonstrates a biological difference between p60^{src} and p91^{fps}. Although recent reports suggest that FSV p140$^{gag-fps}$ is loosely associated with the plasma membrane[12,13], we have not observed this for p91^{fps} in our conditions for cell fractionation (Fig. 4*B*).

As p60^{src} and p91^{fps} are partially homologous[8], we wanted to test the effect of the first 14 amino acids of p60^{src} on an unrelated protein. We constructed RSV genomes in which the *src* gene is replaced by the gene for chimpanzee α-globin[10], or by a fusion of the sequence coding for the first 14 amino acids of p60^{src} and the α-globin gene (the structures of these recombinants are shown in Figs 1, 2). Cells transfected with these DNAs were labelled with ^{3}H-leucine or ^{3}H-myristic acid. Figure 3*B* shows the proteins immunoprecipitated from these cells with anti-α-globin antiserum. Cells transfected with pSR-XD318, the plasmid encoding α-globin, produce a 15K protein, approximately the expected size[10]. Cells transfected with pSR-XD319, the plasmid encoding the *src*–globin fusion protein, produce a 17 K protein. Both of these bands are specific to the DNA used for transfection, and are not seen in immunoprecipitates with normal serum, therefore they probably represent α-globin and the *src*–globin fusion protein, respectively. Only the *src*–globin fusion protein is labelled with ^{3}H-myristic acid.

Peptide sequences that direct post-translational modifications have been described elsewhere[15]. It is probable that the first 14 amino acids of p60^{src} contain a signal for N-myristylation. However, there is no obvious sequence homology between the N-terminal portions of other myristylated proteins[16–19] and that of p60^{src}.

The α-globin protein, and to a lesser extent the *src*–globin fusion protein, are quite unstable in chicken embryo fibroblasts (data not shown), and are therefore difficult to label efficiently with ^{3}H-leucine. We have been unable, therefore, to determine the subcellular localization of the α-globin protein. However, the *src*–globin fusion protein is found primarily in a crude membrane pellet after extraction of cells in isotonic salt (data not shown), which is consistent with the p60^{src} N-terminus having a similar effect on α-globin as it has on p91^{fps} with respect to subcellular localization.

The Harvey sarcoma virus transforming protein, p21^{ras}, is covalently modified with palmitic acid[2], a modification which requires a short C-terminal sequence and correlates with p21 membrane association[20]. Short sequences which target proteins to their appropriate subcellular locations have been described for secreted proteins, mitochondrial proteins, integral membrane proteins, and nuclear proteins[21–25]. The results presented here show that the myristylated N-terminal 14 amino acids of p60^{src} contain a sequence which is sufficient for myristylation, and which may direct p60^{src} and p93$^{src-fps}$ (and possibly the *src*–globin fusion protein) to the plasma membrane.

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1. Krueger, J. G., Garber, E. A. & Goldberg, A. R. *Curr. Topics Microbiol. Immun.* **107**, 51–124 (1983).
2. Sefton, B. M., Trowbridge, I. S., Cooper, J. A. & Scolnick, E. M. *Cell* **31**, 465–474 (1982).
3. Garber, E. A., Krueger, J. G., Hanafusa, H. & Goldberg, A. R. *Nature* **302**, 161–163 (1983).
4. Cross, F. R., Garber, E. A., Pellman, D. & Hanafusa, H. *Molec. cell. Biol.* **4**, 1834–1842 (1984).
5. Buss, J. E. & Sefton, B. M. *J. Virol.* **53**, 7–12 (1984).
6. Schultz, A. M., Henderson, L. E., Oroszlan, S., Garber, E. A. & Hanafusa, H. *Science* **227**, 427–429 (1984).
7. Pellman, D., Garber, E. A., Cross, F. R. & Hanafusa, H. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
8. Shibuya, M. & Hanafusa, H. *Cell* **30**, 787–795 (1982).
9. Foster, D. A. & Hanafusa, H. *J. Virol.* **48**, 744–751 (1983).
10. Liebhaber, S. A. & Begley, K. A. *Nucleic Acids Res.* **11**, 8915–8929 (1983).
11. Feldman, R. A., Wang, E. & Hanafusa, H. *J. Virol.* **45**, 782–791 (1983).
12. Woolford, J. & Beemon, K. *Virology* **135**, 168–180 (1984).
13. Moss, P. *et al. J. Virol.* **52**, 557–565 (1984).
14. Courtneidge, S. A., Levinson, A. D. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3783–3787 (1980).
15. Wold, F. *A. Rev. Biochem.* **50**, 783–814 (1981).
16. Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C. & Titani, K. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6128–6131 (1982).
17. Aitken, A. *et al. FEBS Lett.* **150**, 314–318 (1982).
18. Henderson, L. E., Krutzsch, H. C. & Oroszlan, S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 339–343 (1983).
19. Schultz, A. M. & Oroszlan, S. *Virology* **133**, 431–437 (1984).
20. Willumsen, B. M., Christensen, A., Hubbert, N. L., Papageorge, A. G. & Lowy, D. R. *Nature* **310**, 583–586 (1984).
21. Kreil, G. *A. Rev. Biochem.* **50**, 317–348 (1981).
22. Schatz, G. & Butow, R. A. *Cell* **32**, 316–318 (1983).
23. Hall, M. N., Hereford, L. & Herskowitz, I. *Cell* **36**, 1057–1065 (1984).
24. Kalderon, D., Richardson, W. D., Markham, A. F. & Smith A. E. *Nature* **311**, 33–38 (1984).
25. Walter, P., Gilmore, R. & Blobel, G. *Cell* **38**, 5–8 (1984).
26. Cross, F. R. & Hanafusa, H. *Cell* **34**, 597–607 (1983).
27. Takeya, T. & Hanafusa, H. *J. Virol.* **44**, 12–18 (1982).
28. Mathey-Prevot, B., Hanafusa, H. & Kawai, S. *Cell* **28**, 897–906 (1982).

Specific expression of a foreign β-globin gene in erythroid cells of transgenic mice

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The globin gene family represents an attractive system for the study of gene regulation during mammalian development, as its expression is subject to both tissue-specific and temporal regulation. While many aspects of globin gene structure and expression have been described extensively[1], relatively little is known about the *cis*-acting DNA sequences involved in the developmental regulation of globin gene expression. To begin to experimentally define these regulatory sequences, we have taken the approach of introducing cloned globin genes into the mouse germ line and examining their expression in the resulting transgenic animals[2,3]. Here we describe a series of transgenic mice carrying a hybrid mouse/human adult β-globin gene, several of which express the gene exclusively or predominantly in erythroid tissues. These studies demonstrate that regulatory sequences closely linked to the β-globin gene are sufficient to specify a correct pattern of tissue-specific expression in a developing mouse, when the gene is integrated at a subset of foreign chromosomal positions.

Figure 1 shows a restriction map of the hybrid β-globin gene[4] used in these experiments. A portion of the mouse β-major globin gene and 5′-flanking DNA was included to ensure that any regulatory sequences within this region would be compatible with regulatory factors in mouse erythroid cells, and to thus circumvent possible species-specific differences in globin gene regulation. The 3′ end of the gene was derived from the human β-globin gene, allowing the hybrid gene and its mRNA to be distinguished from the endogenous β-globin genes and their

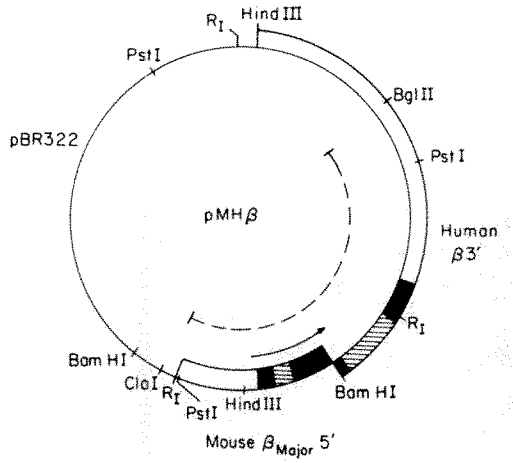


Fig. 1 Restriction map of plasmid pMHβ, containing a hybrid mouse/human β-globin gene. This plasmid was provided by P. Mellon and T. Maniatis, and its construction is described in ref. 4. The plasmid contains the 5′ portion of the mouse β-major globin gene and the 3′ portion of the human β-globin gene, fused at a conserved *Bam*HI site in the second exon of both genes. The clone also includes 1.2 kb of 5′-flanking mouse DNA and 3.8 kb of 3′-flanking human DNA. Solid bars represent exons, hatched bars represent introns, and open bars flanking mouse or human DNA. The broken line indicates the 5.0-kb *Cla*I–*Bgl*II fragment used for microinjection in some experiments.

mRNAs. Mouse zygotes were microinjected[2,5–7] with either the intact circular plasmid pMHβ, or a linear 5.0-kilobase (kb) *Cla*I–*Bgl*II fragment containing the entire gene and 5′-flanking mouse DNA segment, 1.9 kb of 3′ flanking human DNA, and only 350 base pairs (bp) of plasmid DNA (Fig. 1). Ten transgenic mice were produced, three carrying the entire plasmid and seven carrying the *Cla*I–*Bgl*II fragment (Table 1). When mated with normal mice, each of these transgenic mice transmitted the foreign DNA to a fraction of its progeny, and 10 transgenic lines were established. Southern blot analysis (data not shown) revealed that each line carries one or more copies of a 3.4-kb *Pst*I fragment including the β-globin gene, nearly all of the 5′-flanking mouse DNA and 0.5 kb of the 3′-flanking human DNA (Fig. 1, Table 1).

To determine the pattern of expression of the foreign β-globin gene in each transgenic line, we performed a quantitative S_1 nuclease protection assay[8,9] on RNA samples isolated from erythroid and non-erythroid mouse tissues. Mice in four of the lines (46, 49, 75A and 77) were found to express the gene predominantly or exclusively in erythroid tissues; see Fig. 2*a*. The hybridization probe used here derives from the 3′ end of the human β-globin gene, and a 212-nucleotide fragment is protected from S_1 nuclease digestion by either correctly terminated mRNA encoded by the hybrid β-globin gene, or by human β-globin mRNA[4]. For purposes of comparison, only the 212-nucleotide band obtained with each RNA sample is shown (this was the only reproducible band observed). It can be seen that the highest levels of mRNA derived from the hybrid β-globin gene are found in bone marrow and blood cells.

To confirm that the hybrid β-globin mRNA detected in total blood and bone marrow RNA preparations represented expression in erythroid cells, we analysed RNAs from the tissues of several mice that had been injected with phenylhydrazine, a treatment which causes the spleen to become a major site of erythropoiesis[10]. As demonstrated in Fig. 2*b* for mice in lines 77 and 46, the level of hybrid β-globin mRNA in the spleen after phenylhydrazine treatment was equivalent to that in the bone marrow, while in untreated mice (for example, Fig. 2*a*) the level in spleen was much lower.

In some animals, relatively low levels of hybrid β-globin mRNA were detected in RNAs from certain non-erythroid tissues, particularly heart, lung and kidney (Fig. 2*a*, *b*). We

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suspected that much of this mRNA might have derived from blood cells contaminating these tissues rather than from actual transcription of the foreign β-globin gene in non-erythroid cells; this was confirmed by measuring the amount of endogenous β-globin mRNA contaminating different non-erythroid tissues. In general, those non-erythroid tissues containing the highest levels of hybrid β-globin mRNA also contained the highest levels of endogenous mouse β-globin mRNA (an example is shown in Fig. 2*c*). There were a few non-erythroid tissues (testes of a line 49 mouse and heart and kidney of line 75A mice; Fig. 2*a*) in which hybrid β-globin mRNA appeared to result from inappropriate expression of the foreign globin gene at a very low level (less than one mRNA molecule per cell).

The steady-state level of hybrid β-globin mRNA in each mouse RNA sample was estimated by comparing the observed hybridization signals with those obtained using known amounts of human β-globin mRNA (Fig. 2, Table 1). The mRNA levels in erythroid tissues were highest in mice of lines 46 and 49, where they corresponded to 1–2% of the level of endogenous β-globin mRNA (or ~700 molecules of hybrid β-globin mRNA per reticulocyte). Because these mice contained only one gene copy per diploid genome, the level of expression per gene copy was 2–4% of the normal level. Erythroid tissues from mice of lines 75A and 77 contained considerably lower levels of hybrid β-globin mRNA (Table 1).

The transcripts synthesized from the hybrid β-globin gene in erythroid cells of the transgenic mice were found, by primer extension analysis, to be correctly initiated and spliced. A short, ^{32}P-labelled primer from the third exon of the human β-globin gene was annealed to blood RNA from mice in lines 46 and 77, as well as to human β-globin mRNA. When the hybridized primer was extended with reverse transcriptase and dNTPs, the major primer extension products obtained with blood samples from transgenic mice and with human cord blood were indistinguishable in size (Fig. 3).

Gene transfer studies with murine erythroleukaemia (MEL) cells have led to the conclusion that DNA sequences closely linked to, and perhaps within, the β-globin gene are sufficient for the activation of a transfected gene during the terminal events of erythroid cell differentiation[4,11–13]. Our results allow this statement to be extended to include any additional *cis*-acting regulatory sequences which may be needed to activate the gene specifically in the erythroid cells of a developing transgenic mouse. This conclusion must, however, be qualified in several ways. First, maximal transcription of the β-globin gene may require additional regulatory sequences not included on the DNA fragment used in our experiments. The steady-state concentration of the hybrid mRNA in transgenic mouse erythroid cells was no more than a few per cent of the level of endogenous β-globin mRNA, suggesting that the foreign gene was transcribed at a relatively low rate (although an alternative interpretation—a relatively low stability of the hybrid message—has not been eliminated). The highest mRNA levels observed were similar to the mRNA levels (per gene copy) observed when cloned β-globin genes were introduced into MEL cells[4,11–13].

Second, although it seems that the β-globin gene need not be in its normal chromosomal locus to be expressed and regulated, its expression may depend on integration at a specific subset of the available chromosomal positions. Several of the transgenic lines analysed do not express the hybrid β-globin genes in either erythroid or non-erythroid tissues, although they contain at least one intact copy of the gene (Table 1; data not shown). The β-globin gene is not unique in this regard, as several other genes, including metallothionein fusion genes[7,14] and a mouse α-fetoprotein gene[15], are expressed in only a subset of transgenic mouse lines. Occasional cases of low-level expression in inappropriate tissues (for example, mouse lines 15, 49 and 75A; refs 3, 14, 16, 17) may be another consequence of the integration of microinjected genes at abnormal chromosomal positions.

Given the present results, it is surprising that many other cloned α- and β-globin genes are not expressed in erythroid tissues when introduced into the mouse germ line (refs 3, 18, 19; T. Townes *et al.*, personal communication; S. Rusconi,

Table 1 Transgenic mouse lines

Mouse line	DNA injected	Gene copy no.*	Hybrid β-globin mRNA in erythroid tissues: Tissue	(No. of mice analysed)	RNA fraction‡	% Of total β-globin mRNA§
46	5-kb *Cla*I–*Bgl*II fragment	1	Blood	(1)	8×10^{-5}	1.6
			Bone marrow	(3)	5×10^{-6}	0.6
			Spleen†	(1)	5×10^{-6}	0.6
49	5-kb *Cla*I–*Bgl*II fragment	1	Blood	(1)	10^{-4}	2.0
			Bone marrow	(1)	10^{-5}	1.2
75A	5-kb *Cla*I–*Bgl*II fragment	6	Blood	(2)	10^{-6}	0.02
			Bone marrow	(4)	2×10^{-7}	0.02
			Spleen†	(1)	1.5×10^{-7}	0.015
77	5-kb *Cla*I–*Bgl*II fragment	3	Blood	(2)	4×10^{-6}	0.08
			Bone marrow	(4)	8×10^{-7}	0.08
			Spleen†	(2)	10^{-6}	0.1
43	5-kb *Cla*I–*Bgl*II fragment	1				
47	5-kb *Cla*I–*Bgl*II fragment	1				
53	5-kb *Cla*I–*Bgl*II fragment	1				
11	Plasmid pMHβ	9	No expression detected			
16	Plasmid pMHβ	180				
15	Plasmid pMHβ	3	Very low expression ($<10^{-7}$) in all tissues			

Approximately 400–800 circular dimers of the entire plasmid, or 10–30 linear molecules of the *Cla*I–*Bgl*II fragment, were microinjected into the pronuclei of (C57BL/6J×CBA/J) F_2 mouse zygotes. All animals examined were transgenic progeny hemizygous for the hybrid β-globin gene.

* Number of copies of the 3.4-kb *Pst*I fragment per diploid genome in transgenic progeny, determined by densitometry of an autoradiogram of a Southern blot (not shown).

† Animals were treated with phenylhydrazine to induce splenic erythropoiesis, as described previously[3,10].

‡ Fraction of total RNA consisting of hybrid β-globin mRNA, determined by 3′ S_1 nuclease analysis of mouse tissue RNAs, using human cord blood RNA containing a known concentration of human β-globin mRNA as a standard (see, for example, Fig. 2).

§ RNA fraction as a percentage of endogenous mouse β-globin RNA fraction in the same tissue. Values used for mouse β-globin RNA fractions were: blood, 5×10^{-3}; bone marrow, and spleen of phenylhydrazine-treated mice, 9×10^{-4}. These values were determined by RNA titration analysis using a single-stranded probe of known specific activity[25].

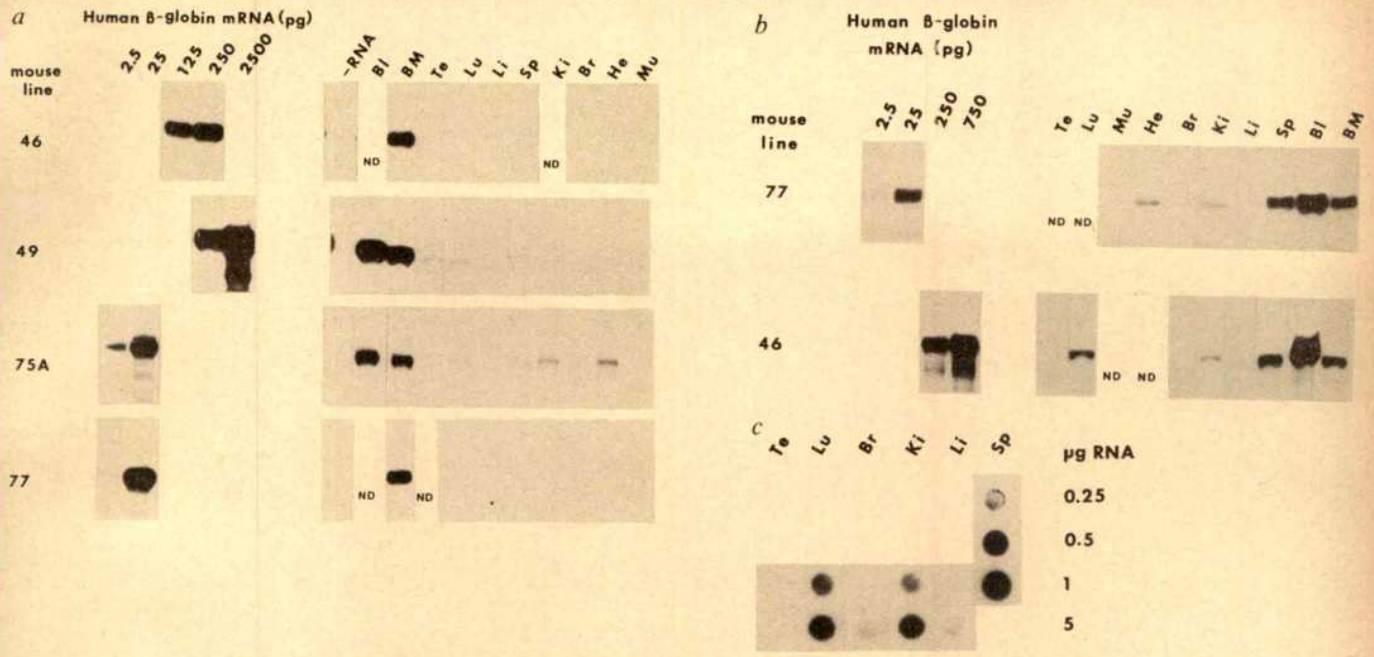


Fig. 2 Specific expression of the hybrid mouse/human β-globin gene in erythroid tissues. *a*, S_1 nuclease analysis of hybrid β-globin mRNA in mouse lines 46, 49, 75A and 77. Total RNA samples from the indicated tissues of a second- or third-generation mouse in each line were analysed by hybridization with a 3′-end-labelled probe derived from the 3′ end of the human β-globin gene, as described elsewhere[4]. For comparison, hybridizations were also performed with human cord blood RNA containing a known amount of human β-globin mRNA. The hybrids were digested with S_1 nuclease and the products fractionated on a 6% polyacrylamide gel containing 7 M urea. Only those regions of the autoradiograms showing the 212-nucleotide probe fragment protected by hybrid mouse/human β-globin mRNA or human β-globin mRNA are shown. The intensities of hybridization signals from the four mice cannot be compared directly because the autoradiograms were exposed for different lengths of time. −RNA, hybridization with only yeast RNA carrier (25 μg); Bl, blood; BM, bone marrow; Te, testis; Lu, lung; Li, liver; Sp, spleen; Ki, Kidney; Br, brain; He, heart; Mu, skeletal muscle. 10 μg of RNA from blood and 25 μg of RNA from all other tissues were used for each hybridization reaction. ND, not determined for the animal whose analysis is shown here. The human 3′ probe does not cross-hybridize with endogenous mouse β-globin mRNA in the conditions used (data not shown). *b*, S_1 nuclease analysis of hybrid β-globin mRNA in tissues of mice treated with phenylhydrazine. A mouse from line 46 and one from line 77 were treated with phenylhydrazine to induce anaemia. Total RNA (25 μg) from each of the indicated tissues was used for hybridization and S_1 nuclease analysis. The specific hybridization signal (212-nucleotide protected fragment) obtained with RNA from each tissue is shown as in *a*. Note the greatly increased level of hybrid mouse/human β-globin mRNA in the spleen RNAs, compared with spleen RNAs from mice in lines 46 and 47 which were not treated with phenylhydrazine. *c*, Dot-blot analysis of endogenous β-globin mRNA contamination in non-erythroid tissues. To estimate the relative degree of blood contamination in non-erythroid tissues, endogenous β-globin mRNA was measured in RNA samples from a phenylhydrazine-treated mouse in line 46 (the same mouse as analysed in *b*). RNAs from the indicated tissues were denatured with formaldehyde, spotted onto a nitrocellulose filter[26], and the filter was hybridized with a ^{32}P-labelled mouse β-major cDNA clone[27]. The two non-erythroid tissues with the highest level of blood contamination (lung and kidney) are the same two tissues that contain the most hybrid β-globin mRNA (*b*).

Methods: Mice were killed by cervical dislocation, organs were dissected and total RNA was isolated as described previously[3,28]. Blood was obtained by heart puncture, and cells were washed once in cold phosphate-buffered saline containing 10 U ml^{-1} heparin before isolation of total RNA. The 0.75-kb *Eco*RI–*Pst*I fragment of the human β-globin gene was 3′-end-labelled with ^{32}P, and strand-separated on a polyacrylamide gel[29] as described[3,4]. The specific activity of the probe was $2–4 \times 10^7$ c.p.m. μg^{-1}, and 4 ng of probe were used for each hybridization reaction. In these conditions of probe excess, the hybridization signal observed (212-nucleotide protected fragment) is proportional to the β-globin mRNA concentration in each reaction. Hybridization was performed at 45 °C for 12–16 h, in 80% formamide, 0.4 M NaCl, 0.04 M PIPES, *p*H 6.8, 1 mM EDTA. Hybrids were mixed with denatured salmon sperm DNA (50 μg ml^{-1}) and digested with 400 U ml^{-1} S_1 nuclease (Sigma) at 15 °C for 1 h. The concentration of β-globin mRNA in the human cord blood RNA was determined (by R_0t analysis; data not shown) to be 1%, in agreement with published estimates of the globin mRNA content of reticulocytes[30].

personal communication). There are several factors that may be responsible for the successful expression of the hybrid β-globin gene in our experiments. First, it is possible that the use of mouse DNA sequences in the hybrid gene construct provided an essential regulatory element that is absent or divergent in the mammalian globin genes used previously. Second, the two transgenic lines which express the hybrid β-globin gene at the highest levels contain a single copy of the gene, rather than the multiple, tandem copies usually observed. The arrangement of exogenous globin genes in tandem arrays may provide an inferior substrate for transcriptional regulation. A different explanation, which we favour, is that plasmid or bacteriophage λ sequences may have an inhibitory effect on β-globin gene expression in transgenic mice; this interpretation is suggested by the fact that all four mouse lines which showed tissue-specific expression contained the hybrid β-globin gene with most of the plasmid sequences removed (Table 1). Furthermore, most of the cloned globin genes that were not expressed in previous experiments were introduced together with prokaryotic vector sequences. This hypothesis remains to be tested definitively.

The results presented here, as well as recent studies demonstrating tissue-specific expression of other microinjected genes[15,16,20–23], indicate that the transgenic mouse system will be useful for elucidating the mechanisms by which genes are activated in specific cell types during development. One important aspect of globin gene regulation which this technique should allow us to study experimentally is the switch from embryonic to adult haemoglobin synthesis during mouse embryogenesis[24]. An analysis of the timing of expression of the mouse/human hybrid β-globin gene has shown that the gene is turned on at the appropriate stage of development for an adult β-globin gene in the mouse (J.M., K.C. and F.C., in preparation). Experiments with additional cloned globin genes, such as embryonic genes or embryonic/adult fusion genes, may allow us to identify *cis*-acting DNA sequences involved in the control of haemoglobin switching.



Fig. 3 Analysis of the 5′ terminus of mouse/human hybrid β-globin mRNA by primer extension. A ^{32}P-labelled single-stranded primer, consisting of a 48-nucleotide *Bst*NI–*Eco*RI fragment from the third exon of the human β-globin gene, was hybridized with human cord blood RNA or blood RNA from transgenic or control mice. The primer was extended with reverse transcriptase and the products denatured and analysed by electrophoresis on a 6% polyacrylamide gel containing 7 M urea. ***a***, Autoradiogram showing analysis of the following RNA samples: Hβ400 and Hβ40, human cord blood RNA containing 400 pg and 40 pg, respectively, of β-globin mRNA; m, molecular weight markers (φX174 DNA digested with *Hin*fI); −RNA, yeast RNA carrier alone (25 μg); 46, total blood RNA (25 μg) from a mouse in line 46; 77, total blood RNA (25 μg) from a mouse in line 77; control, total blood RNA (25 μg) from a mouse not carrying the hybrid mouse/human β-globin gene. The position of the major reverse transcription products expected (418 nucleotides for human β-globin mRNA and 421 nucleotides for the hybrid β-globin mRNA) is indicated by the arrow. No signal was observed when the same reaction was performed with normal mouse blood RNA, showing that the primer is detecting hybrid mouse/human β-globin mRNA rather than endogenous β-globin mRNA in transgenic mouse blood. ***b***, Diagram of the primer extension assay. The heavy line represents the labelled primer and the dotted line the product of reverse transcription after hybridization with human or hybrid mouse/human β-globin mRNA. Preparation of the primer, hybridization and reverse transcription were performed as described in refs 4 and 31, except that 0.4 ng of primer was used for each reaction.

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1. Collins, F. S. & Weissman, S. M. *Prog. Nucleic Acids Res.* **31**, 315–462 (1984).
2. Costantini, F. & Lacy, E. *Nature* **294**, 92–94 (1981).
3. Lacy, E., Roberts, S., Evans, E. P., Burtenshaw, M. D. & Costantini, F. D. *Cell* **34**, 343–358 (1983).
4. Chao, M. V., Mellon, P., Charnay, P., Maniatis, T. & Axel, R. *Cell* **32**, 483–493 (1983).
5. Gordon, J., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. & Ruddle, F. H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7380–7384 (1980).
6. Wagner, E. F., Stewart, T. A. & Mintz, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5016–5020 (1981).
7. Brinster, R. L. *et al. Cell* **27**, 223–231 (1981).
8. Berk, A. J. & Sharp, P. A. *Cell* **12**, 721–732 (1977).
9. Weaver, R. & Weissman, C. *Nucleic Acids Res.* **7**, 1175–1193 (1979).
10. Conkie, D., Kleiman, L., Harrison, P. R. & Paul, J. *Expl Cell Res.* **93**, 315–324 (1975).
11. Wright, S., deBoer, E., Grosveld, F. G. & Flavell, R. A. *Nature* **305**, 333–336 (1983).
12. Charnay, P. *et al. Cell* **38**, 251–263 (1984).
13. Wright, S., Rosenthal, A., Flavell, R. & Grosveld, F. *Cell* **38**, 265–273 (1984).
14. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. *Science* **222**, 809–814 (1983).
15. Krumlauf, R., Hammer, R., Tilghman, S. & Brinster, R. *Molec. cell. Biol.*, (submitted).
16. McKnight, G. S., Kuenzel, E. A., Hammer, R. E. & Brinster, R. L. *Cell* **34**, 335–341 (1983).
17. Jaenisch, R. *et al. Cell* **24**, 519–529 (1981).
18. Stewart, T. A., Wagner, E. F. & Mintz, B. *Science* **217**, 1046–1048 (1982).
19. Humphries, R. K. *et al.* in *Eucaryotic Gene Expression* (eds Kumar, A., Goldstein, A. L. & Bahouney, G. V.) (Plenum, New York, 1983).
20. Brinster, R. L. *et al. Nature* **306**, 332–336 (1983).
21. Storb, U., O'Brien, R. L., McMullen, M. D., Gollahon, K. A. & Brinster, R. L. *Nature* **310**, 238–240 (1984).
22. Grosschedl, R., Weaver, D., Baltimore, D. & Costantini, F. *Cell* **38**, 647–658 (1984).
23. Swift, G. H., Hammer, R. E., MacDonald, R. J. & Brinster, R. L. *Cell* **38**, 639–646 (1984).
24. Russell, E. S. *Adv. Genet.* **20**, 357–459 (1979).
25. Scheller, R. H., Costantini, F. D., Kozlowski, M. R., Britten, R. J. & Davidson, E. H. *Cell* **15**, 189–203 (1978).
26. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
27. Rougeon, F. & Mach, B. *Gene* **1**, 229–239 (1977).
28. Chirgwin, J., Przybyla, A., MacDonald, R. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
29. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
30. Humphries, S., Windass, J. & Williamson, R. *Cell* **7**, 267–277 (1976).
31. Treisman, R., Proudfoot, N. J., Shander, M. & Maniatis, T. *Cell* **29**, 903–911 (1982).

Partial deficiency of erythrocyte spectrin in hereditary spherocytosis

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Hereditary spherocytosis (HS) is a common, clinically heterogeneous haemolytic anaemia[1,2] in which the primary erythrocyte defect is believed to be some abnormality in the spectrin–actin membrane skeleton[3,4], leading to loss of surface membrane[5]. Recessively inherited spectrin deficiency with extreme erythrocyte fragility and spherocytosis has been identified in certain mutant mice[6,7] and two severely anaemic humans[8]. Although suspected, deficiency of spectrin has not been demonstrated in less severe forms of human HS[9]. We now report the quantitation of erythrocyte spectrin by radioimmunoassay. We found that normal erythrocytes contained 240,000 copies of spectrin heterodimer, whereas erythrocytes from 14 patients with a variety of types of HS were all partially deficient in spectrin (range 74,000–200,000 copies), the magnitude of the deficiency correlating with the severity of the disease. Spectrin deficiency of varying degrees is common in HS and probably represents the principal structural defect leading to loss of surface membrane.

Analysis of Coomassie blue-stained SDS-polyacrylamide electrophoresis (PAGE) gels has revealed deficiencies of specific erythrocyte membrane proteins in certain inherited haemolytic anaemias[10]. We have subsequently found that erythrocytes from humans with mild HS have very subtle reductions in spectrin relative to band 3. Figure 1 shows a Coomassie blue-stained SDS-PAGE slab with erythrocyte membranes prepared from a normal individual and four patients with HS. Determination of relative protein concentrations by the pyridine dye elution method[11] showed the amount of spectrin relative to band 3 to be reduced in the HS samples (Fig. 1 legend). Similar membrane analyses were conducted on erythrocytes from more than 100 individuals, including HS patients from 28 different families. We found that erythrocyte membranes from 39 of 43 HS patients showed small but consistent reductions in spectrin relative to band 3, whereas unaffected relatives, patients with other types of anaemia (including warm-reacting autoimmune haemolytic

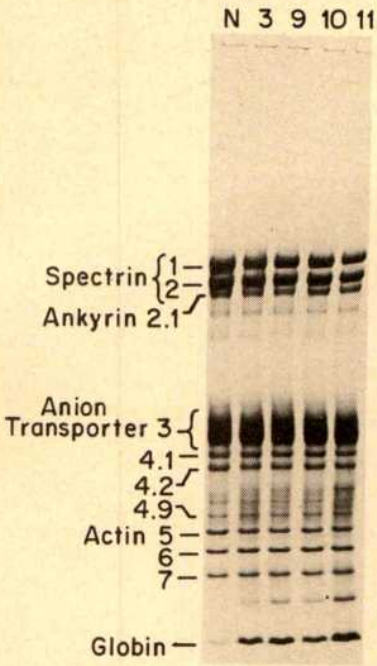


Fig. 1 Coomassie blue-stained SDS-PAGE slab of membranes from a normal individual and four HS patients. Freshly drawn venous blood was washed three times in phosphate-buffered saline (PBS) with removal of the buffy coat. Erythrocytes were lysed and washed repetitively at 0 °C with 7.5 mM sodium phosphate, 1 mM Na-EDTA, 0.2 mM phenylmethylsulphonyl fluoride as described previously[27]. Approximately 25 μg of membrane protein from samples obtained from each of several patients and a control was applied to the top of a 3.5-17% exponential gradient polyacrylamide slab gel and electrophoresed for 3 h at 240 V (ref. 28). Membranes in lane N are from a normal individual and membranes in the other lanes are from patients listed in Table 1. Relative protein concentrations in the spectrin regions of each lane were compared with that in the band 3 region by the pyridine dye elution method[11] and were analysed as the ratios of absorbance at 605 nm of spectrin and band 3: normal = 1.12; patients: 3 = 0.74; 9 = 0.84; 10 = 0.84; 11 = 0.65.

anaemia) and other controls showed no such reduction. Although these analyses indicate that a relative spectrin deficiency commonly exists in HS, they are only semi-quantitative, as this method does not allow determination of the actual number of copies of spectrin per erythrocyte.

We therefore developed a radioimmunoassay to quantify the number of copies of spectrin per erythrocyte. This assay is performed on washed erythrocytes using purified control spectrin as a standard and ^{125}I-labelled pure spectrin as a probe (Fig. 2). Incubation of a known number of washed erythrocytes in 1% SDS at 60 °C results in complete unfolding of membrane proteins, allowing accurate measurement of the actual number of copies per cell by radioimmunoassay[12]. The SDS-lysed erythrocytes were diluted into buffer containing Triton X-100 and albumin to quench the denaturing effect of SDS. Spectrin in these lysates and purified spectrin each competed with ^{125}I-labelled spectrin for binding to immobilized anti-spectrin antibodies, and the profiles of displacement by erythrocyte lysates and by pure spectrin were virtually parallel (Fig. 2). The concentration of purified spectrin and the number of erythrocytes producing 50% displacement of ^{125}I-labelled spectrin were determined by measurements on erythrocytes from each of three normal adults on multiple occasions, and an average of 240,000 copies of spectrin heterodimer per normal erythrocyte was calculated (range 225,000-258,000), reasonably close to the 216,000 copies estimated from Coomassie blue-stained SDS-PAGE gels[13].

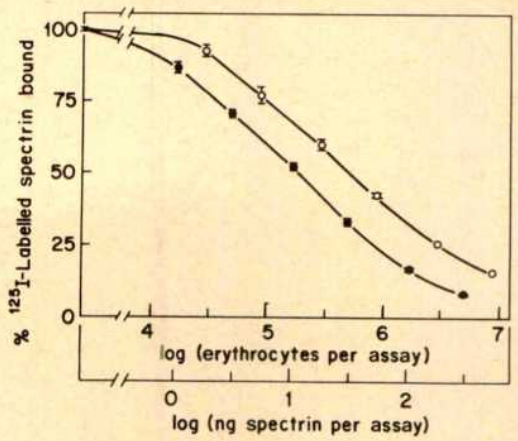


Fig. 2 Radioimmunoassay using ^{125}I-labelled spectrin which is displaced from anti-spectrin antibodies adsorbed onto protein A-bearing staphylococci by spectrin purified from normal erythrocytes (○) or by detergent-solubilized erythrocytes (●).
Methods. Spectrin was purified from normal erythrocytes[27], and the precise protein content was determined from the ultraviolet absorbance spectrum and the extinction coefficient (ref. 29), $E^{1\%}_{1cm}$ 10.7. Pure spectrin was radiolabelled with Na^{125}I to a specific activity of 2×10^7 c.p.m. per μg as described by Hunter and Greenwood[30] and stored at 0 °C at a concentration of 13 μg ml^{-1} in radioimmunoassay buffer (150 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA, 1 mM NaN_3, 1% (v/v) Triton X-100, *p*H 7.5, also containing 1 mg ml^{-1} bovine serum albumin and 5 μg ml^{-1} pancreatic trypsin inhibitor). The immunoadsorbent was prepared from washed protein A-bearing staphylococci and serum from rabbits immunized with human spectrin as described elsewhere[31]. Erythrocytes were washed three times in PBS with removal of the buffy coat and suspended to a haematocrit of 10%. Aliquots of this suspension were removed and counted with a Coulter model B instrument. Aliquots of the erythrocyte suspensions, pure spectrin and ^{125}I-labelled spectrin were separately dissolved and incubated at 60 °C for 10 min in PBS containing 1% (w/v) SDS and then diluted at least 40-fold into radioimmunoassay buffer. The assay was performed in 12×75-mm polystyrene tubes containing 0.2 ml of radioimmunoassay buffer with ~100,000 c.p.m. of ^{125}I-spectrin, varying dilutions of erythrocyte lysate or pure spectrin and a 0.5% (v/v) suspension of antispectrin-staphylococci (diluted ~1:9 with washed staphylococci to reduce the capacity of the immunoadsorbent to precipitate only 40% of the total ^{125}I-spectrin). Samples were shaken gently overnight at 4 °C, then washed with 4 ml of 2 M urea, 1% (v/v) Triton X-100, 0.1 M glycine and the pellets were collected by centrifugation (20 min at 3,500*g*) and analysed for ^{125}I. The final SDS concentration was never more than 0.025% (w/v) and, in the presence of 1% (v/v) Triton X-100, did not interfere with ^{125}I-spectrin binding. Binding of ^{125}I-spectrin to control immunoadsorbents, prepared from preimmune rabbit serum and staphycocci, was negligible and therefore not subtracted. Points represent means ±s.e.m. for triplicates. The content of spectrin in solubilized erythrocytes was determined by calculating the number of cells and the quantity of pure spectrin producing 50% inhibition of ^{125}I-spectrin binding by linear regression of data points in the linear portions of the profiles. A radioimmunoassay using ^{125}I-labelled 43,000-M_r cytoplasmic fragment of band 3, prepared as described by Bennett[27], and immunoadsorbent, prepared from affinity-purified anti-43,000-M_r fragment IgG and protein A-bearing staphylococci, was performed similarly.

The radioimmunoassay can also be modified to measure band 3 content. However, purified band 3 is extremely hydrophobic and unstable, and therefore is neither a good protein to label with ^{125}I nor a stable source of protein standard. Therefore, it was necessary to use an ^{125}I-labelled cytoplasmic fragment of band 3 (relative molecular mass (M_r) 43,000) as a stable, water-soluble probe[14] and normal control erythrocytes as a standard for comparison with HS erythrocytes.

Spectrin and band 3 radioimmunoassays were performed in parallel on erythrocytes from multiple controls as well as HS

patients. The band 3 radioimmunoassay was used, as band 3 is an integral membrane protein, 60–80% of which is not linked directly to the membrane skeleton via ankyrin[15], and it was expected that the unlinked fraction would be relatively preserved even in membranes deficient in membrane skeleton proteins. Table 1 shows the results of these analyses. It is clear that normal individuals of different ages, individuals who had undergone splenectomy for reasons other than anaemia, and patients without HS who had microcytosis and reticulocytosis all had a similar number of copies of spectrin per erythrocyte. Of 14 HS patients studied who had dominant or non-dominant patterns of inheritance and clinical severities ranging from mild to nearly lethal, all had significant reductions in the number of copies of spectrin per erythrocyte. The reductions varied between patients but correlated with the clinical estimation of disease severity, ranging from 200,000 copies per erythrocyte for the least affected to only 74,000 for the most seriously affected. The reduction in the number of copies of spectrin was highly reproducible when measured on multiple occasions on erythrocytes from the same HS patients (interassay variability $<\pm 4\%$). Erythrocyte spectrin content was also found to be similar within families when members with anaemias of comparable clinical severity were compared (members of families B, D and H). Note that the splenectomized member of family D and the relative who had a spleen had erythrocytes with similar spectrin contents. Members within families A and E who had anaemias of different clinical severities were found to have correspondingly different spectrin contents which reflected clinical severity.

Increased sensitivity to hypotonic lysis indicates a relative decrease in surface area to volume[16] and is helpful in establishing diagnosis in HS[1]. Blood samples from the patients listed in Table 1 were quantitatively analysed for shifts in hypotonic lysis profile, and this reflected both spectrin content and disease severity (Fig. 3). Although band 3 content was usually slightly reduced, it was generally less reduced than the spectrin content and was much more variable. It is probable that band 3 content is somewhat more reduced before splenectomy because of the selective removal of certain regions of the membrane by the spleen, possibly related to the process referred to as splenic 'conditioning'. This idea is supported in family D, where the patient without a spleen had significantly more band 3, and higher band 3 contents were also noted in splenectomized non-anaemic individuals. Because of reductions in band 3, the apparent reduction in spectrin relative to band 3 determined from Coomassie blue-stained SDS-PAGE gels always underestimated the true corpuscular spectrin deficiency as measured by radioimmunoassay. The ratio of spectrin to band 3 determined from SDS-PAGE gels (see Fig. 1 legend) is similar to the quotient of the spectrin and band 3 radioimmunoassays (Table 1).

Erythrocytes from patients with all types of HS were found to be deficient in spectrin to varying degrees, whereas erythrocytes from several controls and patients with other types of anaemias were not. A common underlying defect such as partial spectrin deficiency provides a logical explanation for a clinically heterogeneous anaemia in which a reduction in membrane surface area is a recognized common feature. Several observations indicate that the deficiency of spectrin is a primary factor in the pathogenesis of spherocytes. To date, no case of spectrin deficiency has been reported without spherocytes being found in the peripheral blood. Furthermore, when spectrin and actin are stripped from normal erythrocyte membranes, the structure spontaneously disintegrates into tiny vesicles. A partial deficiency in spectrin may result in an underlying membrane skeleton which may not support adequately all regions of the lipid bilayer, leading to loss of small areas of unsupported lipid and untethered integral membrane proteins. If the fragility of HS membranes results primarily from deficiency of spectrin, it might be corrected by addition of spectrin; incorporation of spectrin into erythrocytes of the spectrin-deficient *sph/sph* mutant mice by exchange haemolysis led to partial correction

Table 1 Radioimmunoassay of spectrin and band 3 in erythrocytes

Control individuals					Radioimmunoassay Spectrin Copies per cell	Spectrin % Of control	Band 3 % Of control
Normal adults, 3 persons					240,000	100	100
Normal child					235,000	98	102
Splenectomy without anaemia, 2 persons					241,000	100	119
Hypoplastic anaemia with ovalocytes					250,000	104	
Microcytic anaemia (iron deficiency)					238,000	99	
Microcytosis with reticulocytosis (Hb SC)					255,000	106	108
Patients with hereditary spherocytosis							
Family	Inheritance pattern*	Patient	Clinical severity†	Splenectomy			
A	Dominant	1	Mild		200,000	84	108
		2	Moderate	+	140,000	58	88
		3	Severe	+	122,000	51	86
B	Dominant	4	Mild		192,000	80	69
		5	Mild		187,000	78	69
C	Dominant	6	Mild	+	182,000	76	
D	Dominant	7	Moderate		166,000	69	87
		8	Moderate	+	168,000	70	104
E	Recessive	9	Severe	+	128,000	53	94
		10	Nearly lethal	+	108,000	45	79
F	Non-dominant	11	Nearly lethal	+	80,200	33	68
G	Non-dominant	12	Nearly lethal	+	75,800	32	66
H	Recessive	13‡	Nearly lethal	+	81,600	34	62
		14‡	Nearly lethal	+	74,400	31	69

* Dominant: one affected parent. Non-dominant: both parents clinically normal with no apparent consanguinity. Recessive: both parents clinically normal with known consanguinity.

† Mild: subclinically affected, no anaemia, reticulocytosis ≤2%, splenectomy considered optional. Moderate: anaemia compensated by reticulocytosis before splenectomy which was performed before age 10 yr, no anaemia or reticulocytosis post-splenectomy. Severe: incompletely compensated haemolytic anaemia requiring occasional transfusions before splenectomy performed before age 3 yr, reticulocytosis >2% post-splenectomy. Nearly lethal: dependent on regular transfusions to maintain haematocrit >14% before splenectomy performed in infancy, post-splenectomy reticulocytes 4–10% with haematocrit ~30% in patients 11–14.

‡ Previous evaluations of these patients have been reported.

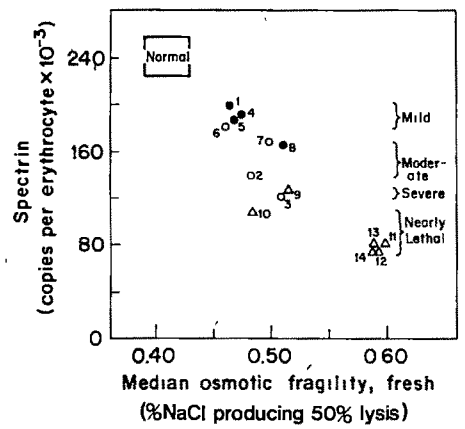


Fig. 3 Correlation of erythrocyte spectrin content and sensitivity to hypotonic lysis. Erythrocyte spectrin content was estimated by radioimmunoassay for each patient and is listed in Table 1 ○, ●, dominant inheritance; △, non-dominant or recessive inheritance; ○, △, previous splenectomy The sensitivity to hypotonic lysis (osmotic fragility) was conducted on fresh blood samples mixed with PBS of concentrations ranging from 0.9% NaCl to distilled water[1]. Median lysis was measured by interpolating between points to estimate the concentration of NaCl at which 50% of cells had lysed. The box at the upper left indicates the range of spectrin determinations on erythrocytes from multiple individuals (Table 1) and the range of median lysis as determined on erythrocytes from multiple normal adults and children

of the mechanical fragility[17]. Reductions in other proteins that bind to spectrin would be expected secondary to deficiency in spectrin, and small but variable reductions in band 3 content were noted by radioimmunoassay as well as small reductions in ankyrin, band 4.1 and actin by analysis of SDS-PAGE gels[8] (for example, Fig. 1). Band 3 deficiency is unlikely to be the primary defect in HS, as this correlated neither with clinical severity nor with osmotic fragility. Small reductions in ankyrin probably result from early degradation of uncomplexed ankyrin, although primary ankyrin defects or deficiencies may exist in certain HS families. A small reduction in band 4.1 is also unlikely to be a primary structural defect in HS, as a 50% deficiency in band 4.1 results, not in HS but in a subclinical form of ovalocytosis[18,19].

The spectrin deficiency in human HS probably results from many genetic defects, with each kindred carrying a different mutation. Probable abnormalities are likely to fall into two groups, a reduced spectrin synthesis due to abnormal gene regulation or unstable mRNA, and inherent protein defects in α- or β-spectrin subunits or ankyrin, leading to defective protein–protein associations, intracellular precipitation or increased susceptibility to proteolysis Bodine and colleagues found spectrin-deficient spherocytic mouse mutants lacking ankyrin (*nb/nb*), with reduced spectrin α-subunit synthesis (*sph/sph*), synthesis of apparently unstable α-subunits (sph^{ha}/sph^{ha}) and reduced spectrin β-subunit synthesis (*ja/ja*)[20]. Erythrocytes from three human families with dominantly inherited spherocytosis have been shown to possess two populations of spectrin, a normal population and a variant population binding defectively to band 4.1 (refs 21, 22). Members of the latter family have been found recently to have a reduction in spectrin relative to band 3 on SDS-PAGE gels (P. Becker and S. Lux, unpublished). The defect in the spectrin-4.1 interaction may result either in reduced spectrin incorporation into the membrane or increased degradation of membrane spectrin, and as expected, it was the variant population that was quantitatively reduced[21,22]. Hereditary pyropoikilocytosis is a rare anaemia[23–25] in which an inherent spectrin defect probably contributes directly to membrane instability[10] but may also lead to secondary spectrin deficiency, while reduced binding of spectrin to ankyrin is another mechanism which could lead to reductions in membrane spectrin (for example, ref. 26). Thus, it is likely that a large number of human genetic defects, although variable in degree, will have the common result of an overall deficiency in spectrin. The studies reported here indicate that spectrin deficiency, from whatever cause, will result in a haemolytic, spherocytic anaemia if the erythrocytes contain less than 200,000 copies of spectrin.

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1 Dacie, J V in *The Haemolytic Anemias Congenital and Acquired* 2nd edn, 82–150 (Grune & Stratton, New York, 1960)
2 Young, L E *Trans Ass Am. Physns* **68**, 141–148 (1955)
3 Bennett, V J *J cell Biochem.* **18**, 49–65 (1982).
4 Cohen, C M *Semin Hemat.* **20**, 141–168 (1983).
5 Cooper, R A & Jandl, J H *J clin Invest* **48**, 736–744 (1969)
6 Greenquist, A C, Shohet, S B & Bernstein, S. E *Blood* **51**, 1149–1155 (1978)
7 Lux, S E, Pease, B, Tomaselli, M B, John, K M & Bernstein, S. E. in *Normal and Abnormal Red Cell Membranes* (eds Lux, S E, Marchesi, V T. & Fox, C F) 463–469 (Liss, New York, 1979)
8 Agre, P, Orringer, E P & Bennett, V *New Engl. J Med* **306**, 1155–1151 (1982)
9 Knowles, W, Marchesi, S & Marchesi, V T *Semin Hemat* **20**, 159–174 (1983)
10 Palek, J. & Lux, S E *Semin. Hemat* **20**, 189–224 (1983)
11 Fenner, C, Traut, R R, Mason, D T & Wikman-Coffelt, J *Analyt Biochem.* **63**, 595–602 (1975)
12 Bennett, V *Nature* **281**, 597–599 (1979)
13 Steck, T L *J Cell Biol* **62**, 1–19 (1974)
14 Bennett, V & Stenbuck, P *J biol Chem* **255**, 6424–6432 (1980)
15 Bennett, V *Biochim. biophys Acta* **689**, 475–484 (1982)
16 Castle, W. B & Daland, G A. *Am. J Physiol.* **120**, 371–383 (1937)
17 Shohet, S B *J clin Invest* **64**, 483–494 (1979)
18 Feo, C J, Fischer, S, Piau, J P, Grange, M J. & Tchernia, G *Nouv Revue fr. Hémat* **22**, 315–325 (1980)
19 Tchernia, G, Mohandas, N. & Shohet, S B *J clin. Invest* **68**, 454–460 (1981).
20 Bodine, D M, Birkenmeier, C S & Barker, J E *Cell* **37**, 721–729 (1984)
21 Goodman, S R, Shiffer, K A, Casoria, L A & Eyster, M E *Blood* **60**, 779–784 (1982).
22 Wolfe, L C, John, K M, Falcone, J C, Byrne, A M & Lux, S E *New Engl. J Med* **307**, 1367–1374 (1982)
23 Lawler, J, Liu, S-C, Palek, J & Prchal, J *J clin. Invest* **70**, 1019–1030 (1982)
24 Knowles, W J *et al. J clin. Invest* **71**, 1867–1877 (1983)
25 Palek, J, Liu, S-C, Liu, P Y, Prchal, J & Castleberry, R P *Blood* **57**, 130–139 (1981)
26 Zail, S S & Coetzer, T L *J clin Invest* **74**, 753–762 (1984)
27 Bennett, V *Meth Enzym* **96**, 313–324 (1983)
28 Fairbanks, G, Steck, T L & Wallach, D F H *Biochemistry* **10**, 2606–2617 (1971)
29 Kam, Z, Josephs, R, Eisenberg, H & Gratzer, W B *Biochemistry* **16**, 5568–5572 (1977)
30 Hunter, W & Greenwood, F *Nature* **194**, 495–496 (1962)
31 O'Keefe, E & Bennett, V *J biol. Chem.* **255**, 561–568 (1980)

Corrigendum

High rates of *in vitro* synthesis of 1,4-β-D-glucan in cell-free preparations from *Phaseolus aureus*

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Nature **311**, 165–167 (1984)

This letter described conditions for high rates of *in vitro* synthesis of 1,4-β-glucan in membrane preparations derived from *Phaseolus aureus*. This was achieved with extracts prepared in polyethylene glycol 4000 and assayed in the presence of Mg^{2+}, Ca^{2+} and cellobiose. The 24% KOH-insoluble glucan product, formed from UDP ^{14}C-glucose, has been characterized as 1,4-β-D-glucan by several criteria, including digestion by purified exocellobiohydrolase and endo-1,4-β-glucanase, methylation analysis and periodate oxidation.

During further studies on the role of cellobiose in this *in vitro* system, more recent analyses of the product (in collaboration with Dr D. P. Delmer) have now revealed largely the formation of 1,3-glucan from UDP-glucose. This finding is puzzling and it is not yet clear why, in our early studies, 1,4-linked glucan was produced, whereas later, under the same conditions, the product was found to contain only 1,3 linkages.

Work is in progress to clarify exactly what conditions are required to obtain reproducible synthesis of cellulose *in vitro* with plant systems.

Pheromone-processing protease of the yeast *Saccharomyces cerevisiae*

A RECENT paper by Mizuno and Matsuo[1] reports the purification from the yeast *Saccharomyces cerevisiae* of a protease with specificity for cleaving at a pair of basic residues within several peptide substrates. The potential importance of such a finding stems from the fact that virtually all eukaryotic peptide hormones and neurosecretory peptides are released from their precursor polypeptides by scission at pairs of basic residues that flank the mature hormone sequences[2]. Indeed, even in a simple eukaryotic microbe like yeast, secreted biologically active peptides, like the mating pheromone α-factor[3-5] and killer toxin[6,7], are excised from their precursors by initial cleavages at pairs of basic residues, namely Lys-Arg.

The activity characterized by Mizuno and Matsuo[1] is soluble and produces Tyr-Gly-Gly-Phe-Leu-Arg (abbreviated leu-enkephalin-Arg) from a larger synthetic substrate (leu-eukephalin-Arg-Arg-Phe-Ala-NH_2). On the basis of this finding, but with no results relevant to the biology of yeast, Mizuno and Matsuo termed this enzyme 'pro-pheromone-convertase Y' and implied that it may be involved in proteolytic maturation of yeast pheromone precursors[1].

We find this reasoning and methodology to be flawed. Recently, we described a mutation (*kex*2) which blocks the proteolytic processing of both prepro-α-factor and prepro-killer toxin and have isolated the normal *KEX*2 gene by recombinant DNA methodology[8]. We demonstrated that *kex*2 mutants lack a membrane-associated protease with specificity for cleaving on the carboxyl side of a pair of basic residues (not between them). Furthermore, cells carrying the *KEX*2 gene on a multi-copy plasmid greatly overproduce this protease. We have observed (ref. 8 and R. Fuller, unpublished results) that the *KEX*2 protease has a strictly neutral *p*H optimum, is completely Ca^{2+}-dependent, is inhibited by heavy metals (for example, Zn^{2+}, Cu^{2+} and Cd^{2+}), by thiol-modifying reagents and leupeptin, but is not sensitive to inactivation by serine protease inhibitors (for example, phenylmethylsulphonyl fluoride, PMSF). In contrast, the activity described by Mizuno and Matsuo has a broad *p*H optimum, is insensitive to chelators, resistant to sulphhydryl-directed reagents and unaffected by leupeptin, but is inhibited by several classical serine protease inhibitors (including PMSF)[1]. Finally, the DNA sequence of the *KEX*2 gene (A. Brake, unpublished results) predicts a polypeptide of relative molecular mass (M_r) 90,000 with a single, striking, membrane-spanning domain; the protein purified by Mizuno and Matsuo[1] was reported to be of M_r ~43,000. Clearly, the *KEX*2 protease and the protein described by Mizuno and Matuo[1] are quite different enzymes

The yield and specific activity of the preparation obtained by Mizuno and Matsuo were not given in their report. As best as can be calculated from the data provided, the final yield was only 2% or less of the initial activity. Given that one of the major neutral PMSF-sensitive vacuole-associated proteases of yeast, proteinase B, has been obtained as a soluble polypeptide of M_r ~41,000 when no precautions were taken to prevent proteolysis during purification[9] (Mizuno and Matsuo added no protease inhibitors to their buffers for obvious reasons), and considering that purified proteinase B will cleave a synthetic substrate, Leu-Trp-Met-Arg-Phe-Ala (somewhat similar to that used by Mizuno and Matsuo), between the Arg and Phe[10], it is possible that the activity described by Mizuno and Matsuo is a proteolytic fragment of proteinase B. This possibility is testable readily because there are available both antibodies directed against proteinase B[11] and mutations in its structural gene (*PRB*1)[12]. Also, it should be noted that Mizuno and Matsuo generated cell homogenates by lysing whole cells with Zymolyase 60,000, a commercial preparation of secreted hydrolytic enzymes from the bacterium *Oerskovia xanthineolytica* (formerly classified as *Arthobacter luteus*). Hence, the activity described by Mizuno and Matsuo and attributed to yeast could actually result from contamination by the PMSF-sensitive bacterial protease known to be present in preparations of Zymolyase[13,14] Again, this possibility could have been eliminated if the Zymolyase treatment had been performed in osmotically stabilizing medium to generate spheroplasts and if the spheroplasts had been washed thoroughly before cell lysis.

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1 Mizuno, K & Matsuo, H *Nature* 309, 558-560 (1984)
2 Herbert, E & Uhler, M *Cell* 30, 1-2 (1982)
3 Kurjan, J & Herskowitz, I *Cell* 30, 933-943 (1982)
4 Julius, D, Blair, L, Brake, A, Sprague, G & Thorner, J *Cell* 32, 839-852 (1983)
5 Julius, D, Schekman, R & Thorner, J *Cell* 36, 309-318 (1984)
6 Bostian, K *et al. Cell* 36, 741-751 (1984)
7 Skipper, N, Thomas, D & Lau, P *EMBO J* 3, 107-111 (1984)
8 Julius, D, Brake, A, Blair, L, Kunisawa, R & Thorner, J *Cell* 37, 1075-1089 (1984)
9 Ulane, R & Cabib, E *J biol Chem.* 251, 3367-3374 (1976)
10 Kominami, E, Hoffschulte, H, Leuschel, L, Mauer, K & Holzer, H *Biochim biophys Acta* 661, 136-141 (1981)
11 Mechler, B, Müller, M, Müller, H, Meussdoerffer, F & Wolf, D. *J biol Chem.* 257, 11203-11206 (1982)
12 Zubenko, G, Mitchell, A & Jones, E W *Genetics* 96, 137-146 (1980)
13 Scott, J & Schekman, R *J Bact* 142, 414-423 (1980)
14 Scott, J thesis, Univ California, Berkeley (1980)

MIZUNO AND MATSUO REPLY—As observed in three distinct precursors of enkephalin, pro-opiocortin and pro-enkephalin A and B, the enkephalin units to be processed are flanked by paired basic residues such as Lys-Lys, Lys-Arg, Arg-Arg and Arg-Lys, which are thought to be a typical processing signal. This suggests strongly that proteases specific to paired basic residues may be involved in enkephalin processing. Our knowledge of the processing events from the precursor to the mature peptides, however, is limited and so far, candidate enzymes have not been identified[1,2]. Hence, the suggestion that the proteolytic processing at the paired basic signals in the precursors occurs by sequential actions of trypsin-like and carboxypeptidase B-like proteases is only tentative.

S. cerevisiae α-cells synthesize and secrete α-mating factor, which, when processed from a larger precursor, involves cleavage at a paired basic signal, that is, Lys-Arg. Therefore, we have chosen these cells as a simple model system for the study of enkephalin processing and have identified in the cell lysates a novel protease which recognizes specifically and cleaves the peptide bond between paired basic residues[3]. The enzyme, of M_r~43,000, cleaves various substrates related to pro-enkephalins between paired basic residues, but not at single basic residues. Its unique substrate specificity suggests that the enzyme is involved in pro-pheromone processing[3]. In order to clarify the processing mechanism, it is important that the candidate proteases be characterized in the various peptide-hormone-producing cells, even though it is unknown whether the enzyme really participates in the processing. Several investigators including us have looked for pro-hormone-converting enzymes in the secretory granules from anglerfish pancreatic islets[4], rat neurointermediate lobe[5] and bovine adrenomedullary gland[6,7]. Protease activities converting pro-insulin, pro-glucagon, pro-somatostatin and pro-opiocortin to the corresponding mature hormones were found. Our enzyme purified from yeast, however, is the only protease with specificity for a paired basic signal that has ever been isolated to homogeneity. We are convinced that our approach, which is distinct from Thorner's[8], is necessary for elucidating the as yet unknown mechanism of pro-hormone processing.

We believe that our enzyme is neither a proteolytic fragment of proteinase B nor a contaminant from Zymolyase. Proteinase B is inhibited by 90% by thiol reagent, *p*-chloromercuribenzoic acid (pCMB), at 0.93 μM, hydrolyses the peptide Leu-Trp-Met-Arg-Phe-Ala at the Arg-Phe bond and shows both chymotrypsin-like and trypsin-like specificity[9]. In contrast, our enzyme is not inhibited by

pCMB at 1 mM and does not cleave the peptides at the sites of single basic residues (for example, Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH_2)[3], indicating that our enzyme is not related to proteinase B. The fact that our enzyme was identified also in cell extracts prepared mechanically by Dyno-Mill treatment (not enzymatically with Zymolyase) excludes the possibility that our enzyme resulted from contamination of the Zymolyase preparation by the bacterial protease.

At present, nobody has a clear idea of how many different enzymes are involved in the processing, whether each hormone system has its own unique enzymes, or whether several generic enzymes accomplish most of the known cleavages. Therefore, identification of the enzyme candidates participating in the processing is important, and our pro-pheromone convertase Y should be compared directly with Thorner's enzyme.

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1 Docherty, K. & Steiner, D F *A. Rev Physiol* **44**, 625-638 (1982)
2 Loh, Y P, Brownstein, M J & Gainer, H *A Rev Neurosci.* **7**, 189-222 (1984)
3. Mizuno, K & Matsuo, H *Nature* **309**, 558-560 (1984)
4 Fletcher, D. J, Quigley, J P, Bauer, G E & Noe, B D *J Cell Biol* **90**, 312-322 (1981)
5 Loh, Y P & Gainer, H *Proc. natn. Acad Sci. USA* **79**, 108-112 (1982)
6 Mizuno, K, Miyata, A, Kangawa, K. & Matsuo, H *Biochem. biophys Res Commun.* **108**, 1235-1242 (1982)
7 Lindberg, I, Yang, H Y T & Costa, E *Biochem biophys Res Commun* **106**, 186-193 (1982).
8 Julius, D, Brake, A, Blair, L, Kunisawa, R & Thorner, J *Cell* **37**, 1075-1089 (1984)
9 Kominami, E, Hoffschulte, H, Leuschel, L., Mater, K & Holzer, H *Biochim biophys Acta* **661**, 136-141 (1981)

Water in the hydrocarbon core of micelles

ARE water molecules excluded from the hydrocarbon core of surfactant micelles, or can they penetrate in-between hydrocarbon chains? Dill *et al.*[1] give an answer based on the contrast variation method in neutron scattering. This method measures the zero-angle limit of the scattered intensity as a function of the scattering length density of the solvent. If their point of view is correct, it provides an important and general technique for studying the hydration and porosity of all colloidal particles. Unfortunately, their claim is based on a misinterpretation of the contrast variation method.

Indeed, for solutions of non-interacting particles, the zero-angle limit of the intensity is a purely thermodynamic quantity, related only to the mass of the dry particle, and contains no information on the arrangement of the constituents within the particle. Formally, it integrates the 'excess' scattering length density with respect to the solvent[2,3], $I(q\to 0) = AN(\Sigma b_i - \rho_s V)^2$, where A is an instrumental constant, N the number of particles, Σb_i the sum of the scattering lengths of all nuclei 'chosen' as belonging to the particle, V the corresponding volume of the particle and ρ_s the scattering length density of the solvent and q the scattering vector. If solvent molecules are included in the particle, they will contribute equally to Σb_i and to $\rho_s V$, unless their specific volume differs substantially from the bulk solvent, which would be extraordinary. Thus, their net effect on the intensity will be zero.

An equivalent formulation calculates the intensity from the 'average' scattering length density $\bar{\rho}$ of the particle; $I = AN(\bar{\rho}V - \rho_s V)^2$ with $\bar{\rho} = (\rho_p v_p + \rho_s v_s)/(v_p + v_s)$ and $V = v_p + v_s$, where v_p is the volume of the dry particle and v_s is that of the solvent included in it. Through simple algebra, this reduces to $I = AN[(\rho_p - \rho_s)v_p]^2$. Hence only the parameters v_p and ρ_p of the dry particle can be measured.

This formalism also applies to the contrast variation method, where the scattering density of the particle is matched by that of the solvent; the matching point will always be obtained for $\rho_s = \rho_p$. Hence, for micelles, it is the scattering density ρ_p of the dry micelle that is measured; solvent molecules in the micelle are not seen by this method.

We know only two ways of measuring solvent penetration in micelles through neutron scattering: one is to go to large scattering vectors (0.6 $Å^{-1}$), where the structure of the micelle can be resolved[4]; the other is to use concentrated solutions of strongly interacting micelles[5], for then the hydration water increases the effective volume fraction, which becomes a thermodynamic quantity measured through $I(0)$. The general conclusion is that bound water is important (eight per headgroup) but is excluded from the core.

Thus, we believe that the author's conclusion is correct for reasons other than those given in their letter; their method does not, however, prove the point. This was also overlooked by others[6], who have concluded that water is excluded from near the ionic headgroups of micelles. If this method was taken at face value for other colloidal systems it could lead to misleading results.

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1 Dill, K. A *et al. Nature* **309**, 42-45 (1984)
2 Jacrot, B *Rep Prog Phys* **39**, 911-953 (1976)
3 Sturmann, H B & Miller, A *J appl. Crystallogr* **11**, 325-345 (1978)
4 Cabane, B, Duplessix, R & Zemb, T in *Surfactants in Solution* (eds Mittal, K & Lindman, B) Vol 1, 373-403 (Plenum, New York, 1984).
5 Hayter, J B & Zemb, T *Chem Phys Lett* **93**, 91-94 (1982)
6 Tabony, J *Molec. Phys* **51**, 975-989 (1984)

CHEN AND DILL REPLY—We agree with Cabane's point regarding the contrast variation technique, mentioned in the first paragraph on page 44 of our article[1]. But we must emphasize that the original paper by Bendedouch *et al.*[2] did contain firm evidence that there is little penetration of water into the micellar core and that substantial hydration occurs at the outer layer of the micelle where the headgroups of the surfactant molecules reside.

Quoting the analysis procedure used in ref. 2, the differential scattering cross-section per unit volume, for a nearly mono-dispersed and nearly spherical system of micelles isotropically dispersed in solution, is

$$\frac{d\Sigma}{d\Omega}(q) = n_p P(q) S(q\sigma) \qquad (1)$$

where n_p is the number of micelles per unit volume calculable from the surfactant concentration and the aggregation number, $P(q)$ the particle structure factor and $S(q\sigma)$ the interparticle structure factor. The information on micellar structure, aggregation number and hydration comes from both factors $P(q)$ and $S(q\sigma)$. $(d\Sigma/d\Omega)(q)$ is the differential cross-section per unit volume.

For experiments that measure the cross-section at 'zero' q and, for small q (such that $q \times \text{size} < 1$), one can write

$$P(q) = P(0)\exp(-q^2 R_g^2/3) \qquad (2)$$

$$P(0) = \int_V d^3 r(\rho(r) - \rho_s) \qquad (3)$$

and

$$R_g^2 = (P(0))^{-1} \int_V d^3 r\, r^2(\rho(r) - \rho_s) \qquad (4)$$

Using standard notation.

The contrast variation technique which is based on measurements of the zero angle intensity, $P(0)$, indeed contains no information on the solvent (water) penetration and the hydration, because in equation (3) the integration over the volume of the micelle (V) involves the difference of scattering length densities of the micelle and the solvent. Analysis of our 'internal contrast variation' data, more accurate than the 'external contrast variation' data, gives an aggregation number $\bar{n} = 78$ and a dry volume per monomer $V_m = 402$ $Å^3$ (refs 2, 3).

The analysis of the finite q data gives, from equation (2), the radius of gyration, R_g, which, by definition in equation (4), is related to the actual volume V of the micelle (as opposed to the dry volume) and hence would give information on the solvent penetration and hydration. Guinier plots of small q data[2] give the same value of $R_g = 15.4$ Å, independently of the degree of deuteration of the tail groups, which means that the scattering of neutrons is dominated by the hydrophobic tail part of LDS (lithium dodecyl sulphate) monomers forming the core of the micelle. If one assumes that the tails form a close-packed ellipsoidal core with

a semi-minor axis, b, equal to the fully stretched tail length, 16.7 Å, the semi-major axis, a, is then determined to be 23.4 Å using 350 Å^3 as the steric volume of the hydrocarbon tail. The dry head group of the monomer would then occupy a volume equal to $V_H = 402 - 350 = 52$ Å^3, which corresponds to a sphere of diameter $l = 4.6$ Å. Thus, the outer layer of the micelle can be assumed to be a shell of thickness l, consisting of 78 head groups plus solvent molecules. Such a model would predict that there are ~9 water molecules in the outer layer for every one of the head group. The radius of gyration calculated from this model is $R_g = 15.2 \pm 0.2$ Å, in agreement with the experimental value. If we now allow for one solvent molecule per monomer in the core, then, taking the volume of the water molecule to be 30 Å^3, a similar calculation would give $R_g = 15.8$ Å, which disagrees with the experimental value[2].

Finally, systematic measurement of the cross-section in concentrated LDS solution[4] allowed us to fit our model equation (1) consistently well. $S(q\sigma)$, thus extracted, unambiguously gives $\sigma = 2(R + l)$, where R is calculated from the volume of a close-packed dry core and l can be anywhere in the range 4.6–5.6 Å, depending on slight variations of the micellar surface charges.

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1 Dill, K A *et al Nature* **309**, 42-45 (1984)
2 Bendedouch, D, Chen, S H & Koehler, W C *J phys Chem* **87**, 153-159 (1983)
3 Chen, S H & Lin, T L in *Methods of Experimental Physics—Neutron Scattering Vol II* (eds Skold, K. & Price, D L) Ch 16 (Academic, New York, 1985)
4 Bendedouch, D, Chen, S H & Koehler, W C *J. phys Chem* **87**, 2621-2628 (1983)

Species variation in sensitivity of general anaesthesia to high pressure

BASED largely on the coincidence in crustacea of lack of pressure reversal of anaesthesia, insensitivity to strychnine and absence of glycine, and on similarities between strychnine- and high pressure-induced convulsions, Smith *et al.*[1] have proposed a hypothesis of general anaesthesia related to glycine receptors Such an interpretation can be challenged on several grounds.

First, the authors' work[1] shows that the relative potencies of a series of general anaesthetics remain constant across a number of species, whether or not they have glycine receptors. Second, binding of strychnine at the glycine receptor in the mammalian central nervous system is unaffected by general anaesthetics[2]. Third, there is abundant neurophysiological data on the lack of interaction between anaesthetics and glycine. Short-latency, glycine-mediated synaptic inhibitions are not enhanced by general anaesthetics, whereas long-latency γ-aminobutyric acid (GABA)-mediated inhibitions are increased in both amplitude and duration[3]. In a variety of *in vivo* and *in vitro* experimental protocols, the inhibitory effects of glycine are not affected by anaesthetic doses that enhance GABA actions[4-6].

As most of the above experiments have concentrated on barbiturates, we have recently examined the effects of two other clinically useful anaesthetics on the mammalian CNS[7,8]. We found no evidence to support the view that perturbation of the glycine system is a common property of general anaesthetics. The dissociative anaesthetic ketamine did not affect responses of neurones to glycine or GABA but did selectively block activation of the neurones by the glutamate analogue, *N*-methylaspartate[7]; correlated with this, ketamine had no clear effect on synaptic inhibitions but did reduce polysynaptic reflexes[8]. When tested on spinal reflexes and inhibitions in decerebrate cats[8], the steroid anaesthetic, alphaxalone, clearly enhanced long-latency GABA-mediated inhibitions but had no effect on short-latency inhibitions, thought on other grounds to be mediated by glycine[9].

It seems, therefore, that general anaesthetics do not influence the operation of glycinergic synapses. It remains possible that an action on the glycine system underlies the effects of high pressure on vertebrates. The absence of pressure-induced hyperactivity in crustacea[1], presumably an evolutionary advantage to subaquatic species, might support this view. In this case, the present models, which assume that pressure and anaesthetics act at the same site, would need to be re-appraised.

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1 Smith, E B *et al Nature* **311**, 56-57 (1984)
2 Snyder, S H & Enna, S J *Adv biochem Psychopharmac* **14**, 81-91 (1975)
3 Weakley, J N *et al Archs int Pharmacodyn* **171**, 385 (1968)
4 Barker, J L & Ransom, B R *J Physiol, Lond* **280**, 355-372 (1978)
5 Evans, R H & Hill, R G *Experientia* **34**, 1325 (1978)
6 Lodge, D & Curtis, D R *Neurosci Lett* **8**, 125-129 (1978)
7 Anis, N A *et al Br J Pharmac* **79**, 565-575 (1983)
8 Lodge, D & Anis, N A *Br J Anaesth* **56**, 1143-1151 (1984)
9 Curtis, D R & Johnston, G A R *Ergeon Physiol* **69**, 97-184 (1974)

SMITH *ET AL* REPLY—The eloquent points raised by Lodge are both valid and stimulating. As stated in our paper[1], the finding that the pressure reversal of anaesthesia appears to be linked to strychnine sensitivity can be interpreted in more than one way.

If the extended unitary hypothesis (which presupposes that pressure and all general anaesthetics share a common mechanism) is accepted[2], our observations point to strychnine-sensitive processes as an important element in the 'loss of consciousness' in vertebrates. The most widely accepted view would then implicate glycinergic processes. Implicit in this view is that the loss of swimming activity in shrimps, at comparable anaesthetic doses, must arise via a different mechanism. Indeed, our additional finding that shrimps appear to have a dose-response curve that is less steep than that observed for anaesthesia in vertebrates would be consistent with this view.

However, we cannot exclude the possibility that the pressure reversal of anaesthesia could arise from an independent action of pressure resulting in a physiological rather than a pharmacological antagonism of anaesthesia; this is compatible with the view of Lodge, who quite rightly points out that there is no direct evidence that general anaesthetics potentiate the action of glycine-mediated inhibition, although it has been demonstrated that the anaesthetics ketamine and althesin confer substantial protection against strychnine[3]. However, if Lodge's work, at the level of the spinal cord, is considered relevant to loss of consciousness which results from the action of anaesthetics at higher levels within the neuro-axis, then the striking diversity of responses to different agents must lead to a rejection of the unitary hypothesis. This implies that different anaesthetics can act by different mechanisms. Any rejection of such a powerful generalization—the unitary hypothesis—that has provided the key to interpreting the common effects of a disparate group of anaesthetic substances, will require an unequivocal body of evidence.

Whichever interpretation of our results is correct, both are incompatible with present thinking—that general anaesthetics act by inducing a general perturbation of cellular membranes—and we believe that models involving more specific molecular interactions are more appropriate.

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1 Smith, E B *et al Nature* **311**, 56-67 (1984)
2 Miller, J C & Miller, K W. in *Physiological and Pharmacological Biochemistry* Vol 12 (ed Blaschko, H K F) 33-75 (Butterworths, London, 1975)
3 Wardley-Smith, B, Little, H J & Halsey, M J *Br J Anaesth* **55**, 235-236 (1983)



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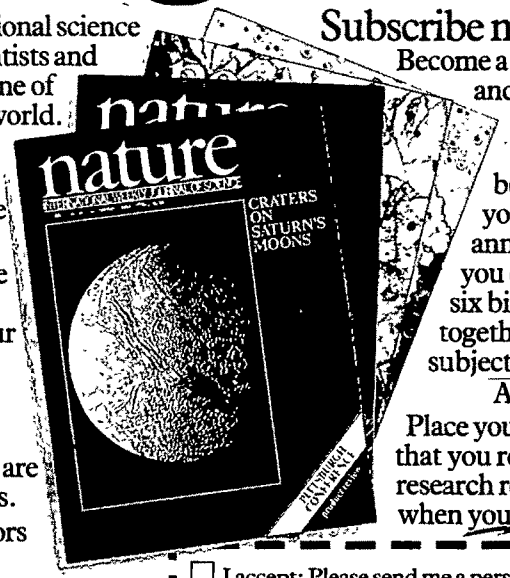
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METHODS IN MICROBIOLOGY

Plasmid Technology

Edited by P. M. BENNETT and J. GRINSTED,
University of Bristol Medical School, England

ISBN: 0-12-521517-7/December 1984 336 pp., $35.00/£25.00

The ability to isolate and manipulate bacterial plasmids has contributed much to the explosive growth of molecular genetics. This volume provides a valuable introduction to the techniques used in plasmid biology. A brief description of plasmids and their importance to bacteria is presented in the first chapter. Subsequent contributions consider experimental approaches to the assignment of genes to plasmids, the transfer of plasmids from one cell to another, the isolation of plasmid DNA, and the physical and genetic characterisation of plasmids. The final chapter discusses one method of DNA sequencing. This work will be vital to all those wishing to exploit plasmid techniques. Experienced workers will also find much of interest.

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REPAIRABLE LESIONS IN MICROORGANISMS

Edited by Dr. A. Hurst and Dr. A. Nasim
1985, 352pp., $55.00/£38.00 (UK only), ISBN 0.12.362690.0

This book presents a comprehensive account of the complexities of DNA lesions caused by a wide variety of specific environmental agents.

CONTENTS: *Introduction: Environmentally Induced DNA Lesions and Their Biological Consequences,* **M.C. Paterson and N.E. Gentner.** *Damage and Repair from Ionizing Radiation,* **N.E. Gentner and M.C. Paterson.** *Damage and Repair from Non-Ionizing Radiations,* **R. Tyrrell.** *Repair Mechanisms and Mutagenesis in Microorganisms,* **M.A. Hannan and A. Nasim.** *Base Modifications and Their Repair,* **S. Riazuddin.** *Microbial Response to Injuries from Host Defense Mechanisms and Antibiotics,* **J.R. Dillon and G.S. Bezanson.** *Osmotic Shock,* **D.B. Wilson.** *Reversible Freeze-Injury,* **B. Ray.** *Reversible Actions of Hydrostatic Pressure and Compressed Gases on Microorganisms,* **R.E. Marquis.** *Reversible Heat Damage,* **A. Hurst. Index.**

GENETICS OF BACTERIA

Edited by J.G. Scaife, D. Leach and A. Galizzi
1985, 272pp., $59.00/£45.00 (UK only), ISBN 0.12.621180.9

The book covers all the major genetic aspects of bacteria, their plasmids, and phages mutation: gene transfer and recombination, the transposition of genetic elements, and the mechanism and control of gene expression. Special emphasis is placed on *Escherichia coli.* In addition, *Bacillus subtilis,* a Gram-positive organism which has unique features as a genetic system, is introduced. For those wishing to study other bacteria a list of helpful references is provided at the end of Chapter One.

A Volume in the Society for General Microbiology Special Publication Series No.13

THE VIRULENCE OF ESCHERICHIA COLI:

Reviews and Methods
Edited by Professor Max Sussman

1985, 473pp., $75.00/£60.00 (UK only), ISBN: 0.12.677520.6

The first part of this book reviews current notions about the virulence determinants and mechanisms of pathogenesis of diseases due to *E. coli.* The genetics of virulence, immunological reactions and the potential of vaccines are also discussed. The second part of the book consists of seventeen chapters that describe methods, otherwise widely dispersed in the literature, for the study of *E. coli* virulence factors.

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Fundamental developments

R.H. Dalitz

Constructing Quarks: A Sociological History of Particle Physics.
By Andrew Pickering.
Edinburgh University Press/University of Chicago Press: 1984. Pp.468. £20, $30.

THE first curious thing about this book is its title. The jacket makes its meaning clear: "scientists are not passive observers . . . of nature, but active constructors of the world of natural phenomena . . .". The second curious thing is that there is nothing inside the book which justifies this conclusion.

The author has a background in theoretical particle physics but is now working as a sociologist of science. His account of the history of experiment and theory in particle physics is both succinct and accurate, and while the book is not written in a popular style it is also not technical; any interested science undergraduate could read it with considerable understanding. One of its threads is to relate the approach and contribution of each of the theoreticians centrally involved to his educational background and previous research experience. These short biographies are factual and interesting; the reader is left to draw his own conclusions. The main theme, however, is that implied by the book's title, as I shall discuss below.

Through the past decade there has been a shift of emphasis in fundamental physics, from hadrons (mesons and baryons) to quarks, largely triggered by the startling discovery of the J/ψ particle in November 1974, although already implicit in our knowledge of hadron spectroscopy (discussed here in Chapter 4) and of deep inelastic scattering of leptons by protons and neutrons (presented in Chapter 5) more than five years earlier. Early in 1974, theoreticians had already predicted from the empirical absence of neutral weak currents involving strangeness change that there should exist a fourth quark c (c = "charm", the first three quarks being u = "up", d = "down" and s = "strange"), estimating its mass to be about 1.5 GeV. The empirical mass for J/ψ was 3.1 GeV and J/ψ soon became understood as a bound state of c with its antiparticle $\bar{c}$, and so provided us with our first "hydrogen atom of quark physics". Particles with non-zero charm, for example mesons with quark structure $(\bar{c}u)$, $(\bar{c}d)$ or $(\bar{c}s)$, were empirically elusive, but the theoreticians insisted on them. The experimenters then worked hard to find them, and their search reached success in 1976, after they adopted a more sophisticated analysis procedure. Chapter 9 gives a good account of these charm discoveries. A fifth quark (b = "bottom") was established in 1977, through the discovery of the Υ (upsilon) particle, and current experiments suggest the existence of a sixth quark (perhaps t = "top"). The masses of these quarks range from several MeV for u and d, to 4.5 GeV for b and about 40 GeV for t.

A deep, elegant and essentially unique theory of the strong interactions of quarks has been developed as a gauge theory based on a symmetry already indicated by the data when we knew only the quarks (u,d,s), a symmetry involving a new degree of freedom for quarks, expressible in terms of a three-dimensional space whose axes are whimsically specified by colours. This theory, discussed in some detail in Chapter 7, is now known as quantum chromodynamics (QCD) and the symmetry as $SU(3)_C$. The gauge field is vector and its quanta are termed gluons. QCD appears to imply that quarks and gluons can only exist within bound states which have no net colour, a constraint known as quark confinement. Since quarks have fractional charge values, they would be readily apparent in the free state, whereas no such objects have yet been established.

Another fundamental step forward was the unification of electromagnetic and weak interactions of quarks and leptons as the electroweak theory, which takes the form of a spontaneously-broken gauge theory based on an SU(2)xU(1) symmetry. This theory was first laid out in 1967 and was proved acceptably finite in 1971. Now known as the Standard Model, in its simplest form it required the existence of some very massive weak bosons ($W^{\pm}$ and Z^0), conceptually partners to the photon, which couple with electromagnetic strength to quark pairs as well as to lepton pairs. This theory also predicted neutral weak currents with no strangeness change; at the prompting of theoreticians (but after a period of uncertainty), the experimenters established the existence of these currents in 1974. The history of gauge theories and electroweak unification is given here at length in Chapter 6; the Standard Model is covered in Chapter 10. The $W^{\pm}$ and Z^0 bosons were identified and measured in 1983, just as this book was going to press, and show excellent accord with the Standard Model.

Although quarks and gluons are confined, quark–quark collisions with high energy- and momentum-transfer occur in very high energy collisions between hadrons. Each secondary quark generates a jet of hadrons which carry its momentum, and the characteristics of quark–quark scattering can be deduced from statistics on these jets. For example, quark–quark scattering is now known to follow Rutherford's Law for spin-½ fermions. Again, weak bosons can decay to a quark–antiquark pair, each generating a quark jet, so leading to phenomena involving correlated jets. In a hadron containing a heavy quark, this quark may transform to a lighter quark with the emission of a pair of leptons, the secondary quark generating a jet of hadrons. There are many possible situations for study in this new area of heavy quark processes.

After reviewing at length the historical development of particle physics, as well as the present scene, the author becomes much exercised by the "incommensurability" of "old physics" and "new physics". The former, typical of the pre-1974 era, is better termed "hadron physics", being the study of the interactions of mesons and baryons, now recognized as "colour-less" composite particles made up of quarks and antiquarks interacting through the gluon field. The latter is better named "quark–lepton physics", where experimental observations are interpreted directly in terms of the fundamental quark–gluon, quark–boson and boson–lepton interactions. This transition to quark–lepton physics has been possible largely because these basic entities appear point-like, so that hard collisions occur at a measurable rate. A similar change of emphasis took place around 1950, when the forefront of fundamental physics moved from nuclear phenomena (the interactions between systems of nucleons) to hadron phenomena (starting with meson–proton scattering), and the accelerators for its study had to be built on the energy scale 1,000 MeV rather than 10 MeV. The simpler phenomena in the physics of hadrons — their detailed spectroscopy, their electromagnetic properties and their interactions at moderate energy — have now been accounted for in considerable detail on the basis of QCD and it is generally anticipated that all of hadron physics, up to moderate energies (say, 10 to 20 GeV), will ultimately be understood in this way, if sufficient theoretical calculation is carried out. For the present, however, we must get on with the study of quark–lepton physics, where the phenomena are much simpler and can teach us more directly about the fundamental interactions.

The author is puzzled at the lack of controversy among physicists about this transition from the study of hadron physics to the study of quark–gluon physics. In the final chapter, entitled "Producing a World", he states: "The history of HEP [high energy physics] can be understood as a communal search for a congenial world: a world which made social sense and in which practice could be socially organised". He concludes that this transition has been a matter of tacit choice, that particle physics today is essentially the result of a conspiracy between experimenters and theoreticians. The theoreticians demand certain phenomena, for their own reasons,

and the experimenters then build equipment sensitive only to those phenomena. As Pickering puts it: "detectors are [now] designed to optimize new physics and to exclude old physics. So specialized have they become that their sole use lies in the investigation of rare phenomena". The experimenters establish what has been required of them, and the theoreticians move on to further demands, in "a social symbiosis of experimental and theoretical practice".

Of course, it must be said here that the theoreticians have had a very good run since about 1970. The gauge principle has proved extraordinarily successful in providing theories which are not *ad hoc* but rooted in deep symmetry principles. But while it is also true that the experimenters have made mistakes, and have had to learn how to deal with background effects which can fog their data on the phenomena they seek to explore, they are not subservient to the theoreticians. In reality, experimenters are cussed individuals, eager to prove the theoreticians wrong whenever possible. In any event, what the author has overlooked is that the experimenters will not be able to find what the theoreticians desire of them, when the theory concerned does not correspond to the real world. Experimenters have the capability of disproving theories, that is the strength of their position, and of course most theories are wrong.

The very direct relationship of the phenomena now seen in the regime of large energy- and momentum-transfer with the underlying theoretical entities and the interactions between them, suggests that particle physics may now have reached a regime of simplicity, as so often hoped for in the past although in a different form. There is no compelling reason why any asymptotic regime of simplicity should exist, of course. This regime stands out only because of the point-like character of the quarks and leptons, relative to the size of about 10^{-13} cm typical for hadrons. As things are, events in this regime are quite rare and it would not have been possible to find them at all amidst the background of events arising from the processes of hadronic physics, if the quark and lepton interactions were suppressed by form factors associated with finite size.

There is, of course, a problem in the minds of many concerning the confinement of quarks and gluons. However, the interpretation of phenomena in terms of entities which we cannot exhibit at the time is not a new situation in physics. The atomic theory of gases was used fruitfully for some decades before a single atom was identified. What *is* new is that the fundamental entities within hadrons are quarks which we cannot hope *ever* to exhibit individually. Are quarks real, in this situation? This is a nice point for philosophical discussion, but in the laboratory there is no doubt at all. Quarks allow us to interpret in simple terms what we see in experiments in this regime. Calculations based on them and their interactions fit the data. They allow us to design new experiments and to decide which measurements are likely to be the most informative. After quite a short period, the active physicist treats them, and thinks of them, as real objects. They have an operational reality for him. On the other hand, these quarks, leptons and bosons are still not necessarily fundamental particles, and the future course of quark–lepton physics is uncertain. Will history run through another cycle and lead us to find that these particles are themselves composite? We see no clear indication for compositeness at present, although there are recent reports of rare anomalous processes in quark–lepton physics for which we have no explanation to hand.

The strange thing about this book is that it is mostly rather good, well-written and engaging, until the author goes off the rails with the idea that the experimenters and theoreticians are making up a world to suit themselves. He is obviously upset that the kind of physics that he learned, "hadron physics", is being ignored in today's experimentation, which does indeed endeavour to eliminate as much of its effects as possible in order to get down to the underlying fundamental physics. Pickering does not seem to have understood the transition which he has been observing, nor to realize that this has happened time and again in the course of physics. However, the sceptical reader will find the book quite rewarding, provided he skips the first and last chapters or takes due warning to read them with caution. □

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Channels defined

Charles F. Stevens

Ionic Channels of Excitable Membranes.
By Bertil Hille.
Sinauer/Blackwell Scientific: 1984. Pp. 426. $32.50, £29.50.

NEUROBIOLOGY has made great strides in the past decade or two, and progress in understanding neuronal electrical activity has been particularly impressive. Bertil Hille's new book provides us with an elegant and lucid account of these recent advances in knowledge about electrical excitability.

What is a channel? When Hodgkin and Huxley showed how the nerve impulse arises from increases in the neuronal membrane's permeability to sodium and to potassium ions, these ions were generally viewed as passing through tiny membrane pores that open in response to changes in the transmembrane voltage. In recognition of the hypothetical nature of these rather mysterious "pores", however, the term "channel" soon came into use; "pore" connotes physical characteristics that the vaguer term "channel" does not and, in the early days when we had no way of directly studying the microscopic processes of ion permeation, the less committal name seemed safer. Over the past ten years, we have discovered that channels are integral membrane proteins in which a microscopic pore opens and closes to regulate the flow of ions across the membrane.

In the old days, two questions about channels were clear. How is gating achieved? And how can a channel select between different ionic species? That is, by what mechanism does a channel open and close its pore, and how can that pore — in a potassium selective channel, for example — let potassium ions (crystal radius = 0.133 nm) pass through while excluding sodium ions (crystal radius = 0.095 nm)? Hille's book gives the modern answers to these classical questions and also deals with more recent issues. What is the structure of channel proteins? How are they synthesized and inserted in the right membranes? How did channels evolve? In what ways are various channel types different and what properties do they share? More specialized sections survey our understanding of chemical and toxin modification of channel gating and of the way in which small molecules of the right shape and size can block pores. Introductory sections cover the required background of physical and chemical principles, and also the classical descriptions of channel behaviour such as the Hodgkin-Huxley model.

The book is written in a clear and crisp style with excellent illustrations and a lot of references (about a thousand of them), ranging from work done in the nineteenth century to articles published in the past five years (about a third of the citations). Chapters are relatively independent, are designed for skipping around in and between, and are arranged in short sections with headlines ("Channels are Needed for Excitation"); "'Hydrated Radius' is a Fuzzy Concept"; "Channel Evolution is Slow").

Not only does Hille's book present information clearly, but it fills an important void: no other systematic and modern treatment of this material is available. And while it is not a Sunday-afternoon-learn-a-bit-about-the-brain book, it does make the modern view of channels available to all neuroscientists — advanced undergraduates, graduate students and scientists who need to know what we understand about channels. Hille has been a leading contributor to research on channels, and has brought the expert's insight, together with the master teacher's skill, to the job of explaining what is known about the basis of neuronal electrical activity.

Charles F. Stevens is a Professor of Physiology at Yale University School of Medicine.

The changing face of the Earth

Robert P. Beckinsale

A Short History of Geomorphology.
By Keith J. Tinkler.
Croom Helm/Littlefield, Adams: 1985. Pp.317. £19.95, $25.

THE compilation of a short, comprehensive and readable history of the study of landforms calls for selective wisdom of the highest class. Keith Tinkler's book has all those virtues, even though nearly one-quarter of it is given over to an excellent bibliography and a rather simple index. The dominant method used is to illustrate particular themes by studying influential publications in some depth to convey an overall view of the subject without sacrificing attention to detail. Necessarily, where possible, the focus falls upon form and process as viewed over time.

The reader is assumed to have at least a nodding acquaintance with geomorphology and its terminology, but these prerequisite demands are small and undergraduates are encouraged also by a brief historical *caveat*. The account moves quickly through classical authors to the Italian Renaissance and so to the long period when the earth sciences, with rare exceptions, were constrained by biblical notions. This period merged into the age of James Hutton and John Playfair who believed that "in nature we have no deficiency of time" and whose thoughts were as complementary as their styles were disparate.

There followed the effect of the Industrial Revolution on scientific thinking, which fostered Charles Lyell, the great uniformitarian, who used existing phenomena as a complete basis for his conclusions on processes (and not surprisingly sometimes showed an unfortunate bias). Before his death in 1875, fluvialism had become more positive and the reality of a recent Ice Age generally accepted, thanks to "a blend of factual investigation and imaginative insight... unparalleled in the history of geomorphology up to that point"(p.119).

Most of the remainder of the text covers geomorphology since the 1880s rightly emphasizing the influence of Eduard Suess who postulated a periodic movement of sea-level, diastrophically controlled and globally synchronous, and of William Morris Davis who popularized stages in river courses and cycles in the development of a variety of landforms. During and after the Second World War allegiance to marine planation and cyclic aspects faded in face of improved technologies and heightened interest in climato-genetic geomorphology and in details of fluvial, coastal and tropical landforms. Since 1960 the shift towards quantification and process studies has quickened and progress has been made in, for example, the understanding of slope development, river flow, ice-sheet dynamics and the significance of physical thresholds. Conceptual and theoretical advances have included the systems theory with its concern with a stable, though not static, equilibrium state.

In the final chapter Tinkler tries to assess future trends, a task which some readers may consider regrettable as this space could have been more profitably devoted to ocean-floor spreading, plate subduction, vulcanism and orogenesis, topics which are almost ignored. But anyone interested in the study of landforms will find this a competent summary which manages to sustain a stimulating commentary throughout its commendably short course. □

Robert P. Beckinsale was formerly a Lecturer in Geography at the University of Oxford.

A chip off the old block

E.N.K. Clarkson

Living Fossils.
Edited by Niles Eldredge and Steven M. Stanley.
Springer-Verlag: 1984. Pp.291. DM138, $54.10.

IN SOME groups of animals and plants the rate of evolutionary change is exceedingly slow, and their modern representatives hardly differ structurally from the earliest members to appear in the fossil record. G.G. Simpson referred to such slow evolution and abnormally long survivorship as bradytely, distinguishing it qualitatively from horotelic (normal) or tachytelic (very rapid) evolutionary change. The survivors of such bradytelic lines are "living fossils", conceived as either specifically adapted to particular niches within a more or less permanent environment or as more than usually tolerant of fluctuating conditions. Lineages which become bradytelic might be "advanced" on their first appearance but lack of environmental stimulus for change would enable them to survive indefinitely.

How valid are Simpson's concepts in the light of the current developments in patterns and processes of macroevolution? Is there anything special about such arrested evolution or is bradytely simply the "slow end" of a normal distribution of evolutionary events?

Eldredge and Stanley, in trying to come to terms with these and related questions, solicited specialist contributions to obtain data on groups which might be, or have been, regarded as living fossils. They requested, for example, information on anatomical similarity of modern forms to their ancient relatives, the time scale involved, ecological specializations, diversity and so on. In other words, how good are the examples of living fossils and in evolutionary terms what does it all mean?

There are 30 separate papers in this collection and two concluding summaries, one by each editor. Over half of the papers are devoted to vertebrates — various mammals, coelacanths, *Amia* and *Polypterus* (though not *Sphenodon*). The interesting question is asked (and answered) as to whether there are any anthropoid living fossils. A great variety of invertebrates are discussed, among them *Neotrigonia*, *Peripatus* and limulines (though not *Lingula*), but also bryozoans, and cephalocarids as living fossils lacking a fossil record. There are, however, no plants, not even *Ginkgo*.

Some authors have given a largely anatomical account of their group, but have usually tried to assess whether the surviving representatives are or are not "good" living fossils. Others have also advanced concepts and theory, often thought provoking. It is, of course, easier to categorize as a living fossil an animal whose similar forebears appeared 500 million years ago, than a type which originated more recently. Even then it may not be easy, for, as Ward, for example, acknowledges in his excellent paper on *Nautilus*, the apparent stasis may only be a factor of low morphological complexity. And again Fisher points out that the sparse fossil record of limulines virtually precludes their evaluation as "good" living fossils. In spite of the very different ways in which the various authors have approached their groups, this volume is a really valuable source of well researched information.

Having collected the evidence, to what conclusions do the editors come as to the reality of bradytely as categorically distinct from other rate-phenomena? Eldredge concludes that there is no strong evidence for bradytely as a separate kind of evolutionary mode. Likewise Stanley suggests that Simpson's three classes, including bradytely (originally developed within a gradualistic framework) tend to lose their value as separate categories when seen from a punctuationist standpoint. Does this imply that, after all, one's own point of view may still be the ultimate determinant, and the question should be left open? Possibly, but few could take exception to the concept that living fossils are simply survivors of long-lived groups which have continued, for a variety of reasons, including those Simpson proposed. □

E.N.K. Clarkson is Reader in Geology at the University of Edinburgh.

New in paperback

● *Ghosts in the Mind's Machine: Creating and Using Images in the Brain* by Stephen Michael Kosslyn. Publisher is W.W. Norton, price is £9.95. For review see *Nature* **310**, 344 (1984).

● *The Youngest Science* by Lewis Thomas. Publisher is Oxford University Press, price is £3.95. For review see *Nature* **302**, 778 (1983).

Mathematics

Algebra Through Practice, 3 Vols. Book 1 Sets, Relations and Mappings. Book 2 Matrices and Vector Spaces. Book 3 Groups, Rings and Fields. By T.S. BLYTH and E.F. ROBERTSON. *Cambridge University Press: 1984 Book 1: pp.97, pbk ISBN 0-521-27285-8; Book 2: pp. 99, pbk ISBN 0-521-27286-6; Book 3: pp. 95, pbk ISBN 0-521-27288-2 Pbk £3.50 each.*

Developments in Statistics, Vol. 4. PARUCHURI R. KRISHNAIAH (ed.). *Academic: 1983. Pp. 287. ISBN 0-12-426604-5. $62.*

Fundamentals of Mathematics. Vols 1 & 2. H. BEHNKE *et al.* (eds). *MIT Press: 1984. Vol. 1: pp. 549, ISBN 0-262-52093-1; Vol. 2: pp. 685, ISBN 0-262-52094-X. £14.25 each.*

Fundamentals of Mathematics. Vol. 3 Analysis. H. BEHNKE *et al.* (eds). *MIT: 1983. Pp. 541. Pbk ISBN 0-262-52095-8 Np.*

Mathematical Magic Show. By MARTIN GARDNER. *Viking: 1984. Pp. 284. ISBN 0-670-80327-8. £9.95.*

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Space Science

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The Universe of Galaxies: Readings from Scientific American PAUL W. HODGE (ed.). *W.H. Freeman: 1984. Pp. 113. Hbk ISBN 0-7167-1675-5; pbk ISBN 0-7167-1676-3. Hbk $20.95; pbk $10.95.*

Physics

The Atomic Nucleus. By J.M. REID. *Manchester University Press: 1984. Pp.279 Pbk ISBN 0-7190-0978-2. Pbk £6.50.*

Bosons in Nuclei. D.H. FENG, S. PITTEL and M VALLIERES (eds). *Wiley: 1984. Pp. 238. Hbk ISBN 0-9971-950-18-9; pbk ISBN 0-9971-950-19-7. £33.90.*

Classical General Relativity. W.B. BONNOR, J.N. ISLAM and M.A.H. MacCALLUM (eds). *Cambridge University Press: 1984. Pp. 269. ISBN 0-521-26747-1. £25, $44.50.*

Coaxial A.C. Bridges. By B.P. KIBBLE and G.H. RAYNER. *Adam Hilger: 1984. Pp. 203. ISBN 0-85274-389-0. £25.*

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Group Theory in Physics, Vol. 1. By J.F. CORNWELL. *Academic: 1984. Pp. 371. ISBN 0-12-189801-6. $75, £49.50.*

Electrochemical Detectors: Fundamental Aspects and Analytical Applications. T.H. RYAN (ed.). *Plenum: 1984. Pp. 172. ISBN 0-306-41727-8 $39.50.*

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From Falling Bodies to Radio Waves: Classical Physicists and Their Discoveries. By EMILIO SEGRE. *W.H. Freeman: 1984. Pp. 298. Hbk ISBN 0-7167-1481-7; pbk ISBN 0-7167-1482-5. Hbk £24.95; pbk £13.95.*

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Superconductivity in Magnetic and Exotic Materials. T. MATSUBARA and A. KOTANI (eds). *Springer-Verlag: 1984. Pp.211. ISBN 3-540-13324-0. DM 65, $23.70.*

Symmetries in Particle Physics. ITZHAK BARS, ALAN CHODOS and CHIA-HSIUNG TZE (eds). *Plenum: 1984. Pp.309. ISBN 0-306-41801-0. $75.*

Theory of NMR Parameters. By I. ANDO and G.A WEBB. *Academic: 1983. Pp.217. ISBN 0-12-056820-9 $52, £30.*

Treatise on Heavy-Ion Science. Vol.1: Elastic and Quasi-Elastic Phenomena. D. ALLAN BROMLEY (ed.). *Plenum: 1984. Pp.753. ISBN 0-306-41571-2. $95.*

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Chemistry

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Comprehensive Treatise of Electrochemistry. Electrodiscs: Experimental Techniques. ERNEST YEAGER *et al.* (eds). *Plenum 1984. Pp 451. ISBN 0-306-41570-4. Np.*

Contemporary Topics in Polymer Science, Vol. 5. E.J. VANDENBERG (ed). *Plenum: 1984. Pp.458. ISBN 0-306-41665-4. $75.*

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Proton and Carbon NMR Spectra of Polymers, Vol. 3. By QUANG-THO PHAM *et al. Wiley: 1984. Pp.677. ISBN 0-471-90394-9. £75.*

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Biological Sciences

Evolutionary Biology, Vol. 18. MAX K. HECHT *et al* (eds). *Plenum:1984 Pp 267. ISBN 0-306-41760-X. $37.50.*

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Fundamentals of Plant Pathology, 2nd Edn. By D. A. ROBERTS and C W. BOOTHROYD. *W.H Freeman:1984 Pp.432 ISBN 0-7167-1505-8. £29.95.*

General

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Scientific Research and Social Goals: Towards a New Development Model. FEDERICO MAYOR (ed.) *Pergamon: 1982. Pp. 236 ISBN 0-08-028118-4 Np.*

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Technological Innovation: University Roles. *The Association of Commonwealth Universities, John Foster House, 36 Gordon Square, London WC1H 0PF: 1984. Pp 431. Pbk ISBN 0-85143-087-2 Pbk £6.*

Total Mean Curvature and Submanifolds of Finite Type. By BANG-YEN CHEN. *World Scientific 1984. Pp 352. Hbk ISBN 9971-966-02-6, pbk ISBN 9971-966-03-4. Hbk np, pbk £15.70.*

nature

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Schools for another century

The British government is rightly seeking to improve the quality of school education. It must first win back the confidence of teachers and then act generously.

How should young people be prepared for life in the brave new world thought likely to begin in 2000 or thereabouts? That question preoccupies educationists in industrialized countries as different as the United States and Japan. Most of the recipes emerging from this introspection entail the assumption that the next century will make greater demands on the numeracy of the general population, which is a fair guess. Governments in the industrialized countries are also conscious that their survivial in recent years has hung on economic competition in the design and manufacture of the products of technological innovation, whence the general objective that educational systems must produce many more young people qualified to engage in this competition. That, too, is a reasonable proposition. The difficulty, as all reformers have discovered, is that it is much easier to specify what the future will require of schools and universities than to arrange matters so that these consequences obtain.

This is the test that must be applied to the torrent of proposals for educational reform now flowing from the British government's Department of Education and Science. Last week, Sir Keith Joseph, the minister in charge, published a policy statement called *Better Schools* whose proposals would transform the way in which schools are organized. This has been quickly followed by a set of detailed proposals for a new public examination to be taken by all British sixteen-year-olds that will break new ground in at least two important ways — the General Certificate of Secondary Education is meant to be a measure of educational attainment for students whose ability spans the whole spectrum, while the grades awarded under the new system will be determined by standards supposed to be objective (and not, as with existing school-level examinations, by competition for a fixed quota of the higher grades). For good measure, the government has also published a policy statement on science education urging that all students should follow science courses from the ages of 5 to 16. Objectively, the new proposals are both enlightened and courageous. But ironically, they have appeared at a time when the British government is in dispute with school teachers over pay. So Sir Keith's rush at reform has won him more enemies than friends. He should not be too surprised.

Improvements

Part of the difficulty is that the minister, like the government of which he is a member, has plainly been forced by the realities of the educational system to abandon some of the prejudices with which it came to office in 1979. British schools were then still adjusting in the wake of the upheavals of earlier decades — the reorganization of secondary schools to provide a comprehensive education within their catchment areas, and the wave of curriculum development that enlivened much teaching but which also threw a disconcerting light on the poverty of the rest. In retrospect, the comprehensive reorganization, socially and even administratively a necessity, was forced through too quickly, with the result that the performance of both teachers and students fell below par. The present goverment's first instinct was to unscramble the reorganization, but by 1979, the omlette was indissoluble. So attention turned to the improvement of educational standards within the publicly supported schools. That is the starting point for *Better Schools*.

There are some things to boast about. The proportions of students leaving secondary schools with qualifications in public examinations have risen over the past two decades, if slowly. The government is able to claim that three-quarters of school-leavers now have graded examination results in at least five subjects. The other side of that coin is that only just over a quarter of them perform creditably, well enough to be able to use their qualifications in persuading employers to offer them jobs. Some of the reasons are spelled out in *Better Schools*. In secondary schools, the planning of the curriculum is too often left to chance. Language-learning is almost universally undervalued. Too many teachers are required to perform beyond the levels for which their training and capacity have equipped them. And so on.

Changes

Complaints such as these have been familiar in British education, as in educational systems elsewhere, for decades. The underlying difficulty centres on the sociology of the school. As in other kinds of educational institutions (universities, for example — see below) the polite conventions of academic life conspire to allow teachers the freedom to teach badly if that is their inclination. In the long run, the only remedy is that schools should become more coherent institutions, places in which professional teachers are involved both with the determination of a school's objectives and with the planning of how flesh should be put on these bones. Too often, in the past, bureaucratic interference from outside, or the waywardness of those in charge of schools, has had the effect of giving teachers the impression that they are merely hired functionaries. One of Sir Keith Joseph's more courageous ambitions is to change all this. Among other things, he plans that professional training courses should be more thorough, that the performance of teachers in posts should be regularly assessed, and that teachers' pay should be linked to performance. Nobody disagrees with these objectives. Why have they landed the minister in hot water?

The most obvious difficulty is that the talk of teacher assessment which has been buzzing about for more than a year has not yet been embodied in a set of tangible proposals that teachers trust. Who will carry out the assessment, and by what criteria? Teachers may be forgiven for asking that these questions should be answered before they entrust their careers to such an innovation, however desirable its effects may be. Yet even now, in last week's flurry of documents on educational policy, it is clear that the government has not made up its mind. There is talk of assessment by local education authorities (which employ teachers) and of the possibility that there may have to be some national scheme backed up by a statutory definition of a teacher's responsibility. But the only level at which the assessment of a teacher's performance can be undertaken sensitively is that of the school. The British government would find its ambitions more easily attainable if schools were given a greater degree of autonomy than they enjoy at present, for then professional teachers would more easily sense the connection there must be between performance, success and security.

Another source of difficulty is that the British government's financial support for schools has become ungenerous. As with British universities, British schools are now kept on tight budgets. The government can claim (as it does in *Better Schools*) that school budgets have not declined as quickly as have school

enrolments, but there are still far too many schools short of resources that they must look to parents for essential provisions of cash. And teachers, unassessed though they may be, are badly paid on rigid salary scales allowing for very little variation in the way in which outstanding members of the profession may be rewarded. The government now seems to be saying that it will devise more imaginative ways of rewarding teachers only when the profession has accepted the need for assessment (and has also swallowed this year's offer on pay). That is too wooden a posture. The government would be better advised to take a more active role in persuading teachers to its convictions. One of the essential ingredients of this process should be an undertaking that the resources spent on school education will be substantially increased as the years go by.

For that, in the long run, is the only way in which the government's ambitions to improve educational standards can be attained. The objective should be that the quality of the publicly supported schools should be comparable with that of the private schools, whose performance is a galling reminder to the public sector of how much improvement there is to make. By comparison, reform of the examinations system cannot contribute as much as would a few tangible demonstrations that it is possible radically to improve the quality of education with Britain's impoverished public sector. For Sir Keith Joseph, the implications should be plain. He cannot hope to bring about his reforms without the willing collaboration of a teaching force whose confidence he has lost. The remedy, painful though it may be, is that he must win them round, chiefly by talk but partly by means of some simple demonstrations of the improvements he rightly wants to see. He may have thought his rash of policy documents would tidy up this important enterprise, but it is only a beginning. □

University business

British universities should seize an opportunity for reform.

THE report of the Jarrett committee on the efficiency of British universities has turned out not to be the fearsome document that some academics had expected. But that does not imply that its modest and sensible proposals will be adopted without difficulty or, for that matter, ignored. The essence of the argument now advanced (see p.394) is that, in spite of the distinctiveness of their social function, there is no reason why universities should not be managed as are other complicated institutions, by the self-conscious planning of their own development and by repeated re-examination of the match (or mismatch) between their resources and their ambitions. On that point, the Jarrett committee is on entirely sure ground. The practical difficulty, during the past four years but probably for some time to come, is that individual universities must draw up strategic plans in ignorance of the resources they will have at their disposal in the future. Crisis management has been the game since the budget cuts of 1981. Planning for uncertainty is now the order of the day.

Come what may, British universities cannot emerge qualitatively unchanged from this period of upheaval, for which Jarrett is merely the latest stimulus. The issue that confronts them is simple, even stark. Faced with shrinking resources, the University Grants Committee, British universities' paymaster, has been compelled to plan to distribute what funds there are in a discriminatory fashion. Something like two-thirds of its pot of gold will be used for the support of teaching, the rest for providing the infrastructure for research. The first task may in due course be done by means of formulae, but the second will entail some necessarily invidious judgements on whether this or that research is academically more interesting, or otherwise more deserving. People (*Nature* included) will be watching the grants committee like hawks to ensure that it does not sacrifice quality in the face of claims (from the government, perhaps) that second-rate work may be more useful, but universities cannot hope to succeed in this environment if they are not equipped to be internally discriminatory (between the good and the less good). That, briefly, is all that Jarrett recommends.

The objections are easily anticipated. Universities everywhere, jealous though they are of their constitutional autonomy, have traditionally shrunk from suggestions that they should apply yardsticks to the quality of the work their people do. It is a painful process, but also one in danger of being corrupted by the special influence of powerful members of a faculty, more able to fight for their own corners than they deserve to be. There will also be cries that attempts to discriminate between different fields of research by the allocation of resources will be an offence against academic freedom, which is a beguiling half-truth. For academic freedom implies merely that an academic should be free to pursue the goals he judges to be worthwhile, not that he should be equipped to do so on some standard scale. Yet in practice, the difficulties of deciding these contentious questions within an exclusively academic body are so great that academics should themselves recognize that it will be simplest if the responsibility can be shared with the university's governing body. That is the logic of the Jarrett recommendation. Nobody should think that it will be easily effected.

Much the same is true of the proposal that members of academic staffs at British universities should be regularly subjected to formal appraisal of performance as are, for example, people who work in business or in the civil service. The Jarrett report remarks that appraisal of a person's performance in academic life now happens only accidentally, perhaps at the end of a probationary period or at promotion or relocation. (It might have added that the criteria used on these occasions are often too narrow, or too unambitious.) Again there will be fears that a formal requirement that senior academics should tell their junior colleagues how they rate will interfere with academic freedom, but this is also mistaken. Jarrett might have made more of the positive benefits of sensitive assessment. As things are, too many British universities wedded to the notion that young academics will either sink or swim are allowing their indifference to the careers of new recruits to become a means of wasting talent.

Most British universities will no doubt read the writing on the wall, and bite the bullets Jarrett offers. What, however, will happen to those that are constitutionally incapable of responding? Oxford and Cambridge, which are not merely autonomous but which are self-governing to an embarrassing degree, may find themselves having to vote away some fo their high-level mob rule. The University of London, by any standards the largest in the United Kingdom, will be in greater difficulties. With its federal structure, the obvious danger is that the central administration will blunt the efforts of its constituent parts to improve their capacity to make decisions. The simplest solution is to allow these parts, all of them now larger than many other British universities, to deal directly with the grants committee. Jarrett's management principles require no less.

Whether reforms along these lines will serve the intended purpose does not, unfortunately, rest with the universities, or even with the grants committee. Increasingly, it is the government that determines policy, but on a short-term basis. The first round of budget cuts decreed in 1980 was based on the doctrine that higher education must bear its share of the general reduction of public expenditure then planned; in the event, it cost the government an unplanned £80 million to send 5,000 academics into early retirement. Now there are budget numbers for the three years ahead, but nobody has explained how the university system is to manage the further contraction they imply. And the government is still silent on the issue of how many students should be admitted to British universities, for what purposes and with what support. The general exhortation that universities and industry should work more closely together is known to have financial implications still undefined. All these questions were supposed by now to have been clarified in a policy paper from the British government, whose publication has been imminent for nearly a year. Sir Keith Joseph, the minister responsible, may have had other things on his mind (see above), but his apparently endless delay in saying what he wants of universities is also bad management of a kind. □

US universities

Now even the Texans are short of cash

Austin, Texas

TEXAN ambition is legendary, and the state's two major campuses, the University of Texas (UT) at Austin and Texas A & M University at College Station, certainly fit into that mould. Both aspire to be counted among the country's top research institutions before the end of the century, and both can point to impressive steps already taken towards that goal, including the well-publicised poaching of academic superstars from better-known and more widely celebrated institutions. But a state budget crisis is casting a shadow over the universities' finances — both are state supported — and faculty recruitment and teaching programmes may suffer as a result.

The Texan economy has been built on oil. Since oil prices started to fall two years ago there have been growing numbers of business failures, and property prices have fallen, sometimes dramatically. State tax revenues have also fallen, and because the state legislature is constitutionally prevented from running a budget deficit it now has to find ways of reducing spending by almost $1,000 million (the alternative, raising tax rates, seems too unpopular even to contemplate in Texas). The first proposal for achieving this saving, drafted against a deadline and hardly intended to be taken seriously, would have done so entirely by reducing the state's higher education budget by a startling 29 per cent. The most recent proposals are more moderate — it seems to be agreed that the 1986 higher education appropriation should not be less than 96 per cent of that for 1985 — but the state House of Representatives appropriations committee has nevertheless approved a plan to close down the UT Permian Basin campus and the A & M campus at Galveston. While this will not be the last word on the plan, it indicates how seriously the crisis is now being taken.

Many fear that even small cutbacks will damage the Texas "can do" reputation, which is held to be the major reason why both universities have been so successful in building first class academic departments. Eight UT Austin PhD programmes were ranked among the top ten in the country by a recent national survey, and A & M claims to be among the top 20 US universities in research expenditure. The chancellor of the UT system is on record as saying that support at 96 per cent of 1985 levels will be insufficient to keep academic programmes at their current levels, however, and the university is still pressing for a 4.9 per cent increase. The funding decisions by the legislature are supposed by law to be decided by midnight of 31 May; this year many expect the legislature to resort to the device (used on some previous occasions) of literally turning back the chamber's clock to gain extra time.

The state budget crunch is doubly unfortunate because it comes at the same time as legislative changes that will reduce the benefits accruing to UT Austin and Texas A & M from a massive land endowment granted by the state last century. The 2 million acres of west Texas that were set aside to support scholarship were later found to lie above substantial oil deposits, and it is principally the oil that has supported the extraordinary expansion of both institutions since the 1920s. Royalties are channelled into a Permanent University Fund, now worth around $2,000 million; interest from the permanent fund constitutes the Available University Fund, amounting to about $150 million a year. The universities use the permanent fund to guarantee loans for new building construction, and interest on this bonded indebtedness is the first call made on the available fund.

In a ballot last November, the state increased the number of campuses eligible to use the Permanent University Fund to guarantee loans, to include virtually all UT and A & M campuses; this privilege had previously extended only to a few sites, principally the large College Station and Austin campuses. The result will be that more of the available fund will be tied up servicing debts and so proportionately less will be available for other improvements at the main campuses.

Despite the squeeze, both universities have more to be cheerful about than most: UT's total endowments are by some reckonings worth more even that those of Harvard, generally thought to be the wealthiest university in the country. And the president of Texas A & M, Frank Vandiver, is adamant that state budget problems will not impede plans to recruit top quality faculty: top salaries can be paid to attract the right people (there are two faculty members earning over $100,000 a year) can be paid to attract the right people, and, even more important, the university will provide buildings and equipment beyond the means of many institutions. The success of A & M in becoming the scientific headquarters of the Ocean Drilling Program is ascribed largely to Vandiver's personal commitment to provide $5 million of university funds for a new building for the programme, the first on the university's research park. A & M is also making a strong pitch to attract the proposed Superconducting Supercollider to Texas, to which end it has already devoted $1 million.

At UT Austin, recruitment is now under way to fill 32 new scientific professorial chairs, each carrying an endowment of $1 million. Last year, the university accepted $16 million in private donations for the chairs and was able to match this sum from the Available University Fund; the first appointments have now been announced. The 32 chairs will be in natural sciences and engineering, with a strong emphasis on molecular biology and materials sciences, reflecting the university's desire to serve the economic needs of society, according to Austin Gleeson, dean of the college of natural sciences. Some faculties outside the favoured areas are resentful of what they see as a lack of consultation by the university on where the money should be spent. But Gerhard Fonken, Dean of academic affairs, argues that when a single gift of $8 million is being discussed it is well to bear the donor's wishes in mind. The first of the 32 appointments have recently been announced, but already there are worrying signs that some scientists who have been approached are being deterred by reports about the state legislature's early proposals for higher education spending. The view at Austin is still firmly optimistic; talk of faculty lay-offs is dismissed as scaremongering. **Tim Beardsley**

Perils of secrecy

Washington

THE Office of Technology Assesment (OTA), which exists to advise Congress on technical matters, has discovered one of the pitfalls of working with classified documents. The office was recently commissioned by a congressional committee to prepare a report on "C³I" — command, control, communications and intelligence of nuclear forces. OTA's researcher was provided with the appropriate security clearances; the final report was to be classified.

After poring over Department of Defense (DoD) documents, OTA's researcher wrote his report and submitted it to DoD for security review. DoD promptly slapped on it a classification even higher than the level for which the researcher had had clearance. The classification is also higher than congressmen are ever allowed. So now neither OTA nor the committee that commissioned the study is allowed to read it.

The explanation is that the OTA researcher apparently pulled together enough top secret and "compartmentalized" data (one step above top secret — those with access to any given compartment are not given access to any others or even permitted to know what other compartments exist) to reproduce some of DoD's most secret data on nuclear war-fighting plans, which have a security classification all their own.

OTA officials have suggested outfitting their researcher with a blindfold to allow him to remove the offending passages.

Stephen Budiansky

British universities

Better management demanded

BRITISH universities are in for another shake-up, this time a revolution engendered from within. So much is clear from the report published earlier this week of the committee under Sir Alex Jarrett set up a year ago to give the Committee of Vice-Chancellors and Principals advice on the effective management of universities.

The study now completed has been an unusual exercise, in which individual academics collaborated with professional management consultants and firms of auditors to look into the efficiency of six institutions (the universities of Edinburgh, Essex, Loughborough, Nottingham, Sheffield and University College, London). The report now published is that of the steering committee for the separate studies, and seeks to emphasize some general conclusions reached.

The report seems to accept that the financial deprivation of the British university system will continue, and perhaps even intensify. The committee notes that the hope that the severe cuts of 1981 (effectively a reduction of budgets by 17 per cent over three years) have not been followed by the once-promised "level funding" but by a period during which central government support for universities has been fixed in cash terms that do not adequately compensate for inflation.

The committee notes that, during this period, the British government has also taken steps to influence the pattern of university development, both by the encouragements of some kinds of academic developments and by limitations of student numbers. The report pleads that, in the interests of efficiency, the government should make clear its intentions towards the British university system and that it should look for some way of enabling universities to plan more than one year ahead. Specifically, the committee asks that the government should be prepared to meet the "whole or the greater part" of the cost of such academic redundancy as may be needed in the future.

Starting from the view that change within universities will be further stimulated by the determination of the University Grants Committee to distribute funds more selectively in future, the committee argues for more decisive management within universities. It says that governing bodies (variously called "councils" and "courts" in the British system) should assume responsibility for strategic planning and that vice-chancellors (also called principals) should be recognized as the chief executives as well as academic leaders of institutions (and be given increased powers to do so when necessary).

Ordinarily, the Jarrett report says, there should be a joint committee of the council and the academic senate, with the vice-chancellor as chairman, to superintend planning and the allocation of resources within a university. At the department level, the report says, steps should be taken to see that the performance of individual academics, as teachers and in research, is regularly assessed. In general, the report asks that universities should prune the numbers of committees dealing with other than academic matters, delegating affairs such as the operation of student residences and the maintainance of buildings to university officials.

The report also asks that the grants committee should itself be more active and articulate, and that it should be more vigilant in monitoring promises made by individual universities (as when, in 1981, universities agreed to close some departments and to run others in collaboration with neighbouring institutions). The Jarrett report also says there should be a review of the grants committee itself, in part so as to tell whether it is sufficiently well-provided with resources for the work it has to do. □

Japanese whaling

Hollow victory for United States?

Tokyo

UNSUSPECTING foreigners out in Roppongi, the nightlife centre of Tokyo, are in for a surprise, for there amongst the flashing neon is a large sign in English, paid for by the All Japan Seaman's Union, reading "Continue the whaling on scientific facts". These words, designed to strike at the heart of logically minded Westerners, may be the last from a dying industry.

Scientific facts or no scientific facts, decisions are rapidly being made about the future of whaling in Japan and, despite last-ditch diplomatic efforts by Japan's Fishery Agency, concessions are having to be made that almost certainly spell doom for the offshore whaling industry.

In December last year, the Japanese government filed a formal objection to the 1984-85 International Whaling Commission (IWC) quota for the minke whale catch in the Antarctic Ocean — minke whaling forming the backbone of the Japanese whaling industry. The IWC quota had been set at 4,224 head, 37 per cent down from the previous year, of which 1,941 head were allocated to Japan. By filing the objection, Japan freed itself from the need to observe any quota at all; soon after, however, Japan announced it would "voluntarily" limit Antarctic minke whaling in the November 1984/March 1985 whaling season to the previous season's levels (3,027 head). This, however, did not appease world opinion sufficiently for the United States to withdraw its threat to invoke the Packwood-Magnusson Amendment, a domestic ruling to cut the fishery catch in US waters of any country that defied IWC quotas. This would mean that Japan's huge fishing quota in the US 200-mile fishing zone would be halved in the first year of defiance and reduced to nothing in the second. To buy time for talks, a further compromise was made: the Japanese Antarctic whaling fleet was ordered to stop catching on reaching the new quota set by IWC and return home.

But the move did little to ease US pressure, and a series of talks between the United States and Japan has led to an ultimatum that Japan must withdraw an objection it filed earlier to the IWC complete ban on commercial whaling that is to begin in 1986. Withdrawal of the objection would effectively mean complete defeat: all Japanese commercial whaling would have to end by 1988. All that the Japanese have so far won from the United States is an extra two years' grace — 1988 rather than 1986 — before they must observe the moratorium. Even this gesture was hotly contested in the courts by US environmentalists. A last-minute attempt to gain further concessions from the United States is under way this week, but the Japanese government has already admitted that little can be expected.

The implication of the Seaman's Union poster is that there is actually no clear scientific reason to stop minke whaling. Even IWC's own scientific committee had not claimed that minke stocks were in danger of extinction. Not surprisingly then, the Japanese see their defeat over whaling as a victory for US environmentalists and a view of the morality of the killing of whales they do not share. Strong emotions have been aroused about the way in which foreign pressure has crushed an ancient industry — indeed the Seaman's Union poster carries a different message in Japanese: "Defend our traditional whaling", and has a picture of a traditional craft with rowers clad in loin-cloths to drive home the point.

US environmentalists have recently tried to ease tension by rewarding cases of Japanese kindness to whales. A baby whale that became entangled in a shore net and ended up still alive at a fish market was recently bought by a group of fifteen Odawara fishermen and returned to the sea. Earth Trust, a US environmental group, then gave them a 20,000 yen reward together with a commendation and a whale sculpture. The fishermen, who had been moved to free the whale because "it looked as though it was shedding tears", will donate the money to African refugees.

The whales may be a lot better off for these developments but the United States may well feel a backlash. The Japanese feel they have been forced to concede and the next issue may be that much harder fought.

David D. Swinbanks

Deep-sea drilling

No cut-price membership

Washington

NATIONS participating in the Ocean Drilling Program (ODP) have come down hard against a suggestion from Britain that it might join the project as a part member, perhaps in collaboration with another country, and so save part of the £2.5 million annual membership fee. The executive committee of Joint Oceanographic Institutions Deep Earth Sampling (JOIDES), which manages ODP, has, while urging the United Kingdom to join, passed a resolution that it would be "neither appropriate nor in the best interests of the program or other full members" for Britain to join except as a full member. JOIDES has also instructed planning committees not to guarantee places for British scientists on future research missions of the new ODP drilling ship, the *JOIDES Resolution*.

Britain became a *de facto* non-member of ODP at the end of January when the British Natural Environment Research Council (NERC) failed to raise sufficient funds in time for a deadline. Britain's representative, John Bowman of NERC, had earlier voted in support of the rule that countries that had not paid up by the end of January would no longer be considered members. Efforts are still under way in Britain, however, to secure the amount needed, and JOIDES officials point out that Britain would be able to rejoin in future years if present efforts fail.

The British unwillingness to find the membership fee has annoyed other full members, because the ODP budget had been planned on the assumption that there would be four full members (besides the United States) during 1985. Other full members are France, West Germany and Canada. Japan is committed to join after September this year, but this was also anticipated in budget planning. The programme will be $2.5 million short next year unless another member is found. Already, purchase of some shore-based equipment has had to be deferred, according to Philip Rabinowitz, director of ODP.

ODP scientists say the British scientific contribution to ODP will also be missed. Britain, for its part, would lose its influence on the committees that plan the cruises of *JOIDES Resolution*. This is likely to cause problems for the independent British Antarctic Survey, which has planned its own work on the assumption that Britain would be a member of ODP and so able to participate in an Antarctic drilling cruise in the Weddell Sea.

Despite JOIDES' firm opposition to part membership for Britain, there are hopes that a consortium of European Science Foundation members may join in partnership with Australia. Britain, still out in the cold, has sought temporary observer membership status like that now enjoyed by Japan, and it seems likely that JOIDES will approve this request. But JOIDES is adamant that observer memberships will not be allowed to continue beyond the end of September. **Tim Beardsley**

The sweet smell of success

Tokyo

BIOTECHNOLOGY may not yet be making much of a return for US venture capitalists, but in Japan it is aleady selling cosmetics just as fast as they can be put on the shelves. Recently launched and already a huge success is cosmetic manufacturers Kanebo's "Bio series" of lipsticks, eye shadow, powder and so on.

What puts the "bio" in the series is that the pigment shiokonin used in the cosmetics, is obtained by large-scale plant cell culture. Until now shiokonin has been extracted from the naturally grown roots of the plant gromwell. But in the new process, gromwell root cells are cultured in 750-litre bioreactors and the pigment recovered from solution (see *Nature* 307, 504; 1984). Interestingly, the scientists who developed plant cell culture at Mitsui Petrochemical Industries had not originally been aiming at the cosmetics market at all. Only when they found that they had developed a working system were they forced to try to find a good product to make with it — cosmetics was one of the last things they hit on. Kanebo is now trying to build on this unexpected success



and claims to have succeeded for the first time in culture of tissue from leaves and stems of aromatic geranium. "Bio" perfumes may thus be just around the corner.

The main impetus behind the increased sales of "bio" products — running at three quarters of a million lipsticks a month and outpacing supply — seems to be the allure of "biotechnology" rather than any major improvement in the product. Other Japanese cosmetics manufacturers are now scrambling to join the bandwagon and find their own products. Alun Anderson

Alternative energy

Fuels from the farm

Venice

RESEARCH in the European Community to produce ethanol fuels from biomass is to be stepped up; this may help to solve the problem of agricultural surpluses and the need for a new fuel blend policy as lead is phased out of fuel, and exhaust emissions are further reduced in the European Community (see *Nature* 28 March, p.304).

The problem of surpluses has injected new urgency in the eight-year-old research proposal, which forms part of a broad high-level Commission strategy by farm commissioner Frans Andriessen. According to Dr Strub, general director of the Commission's solar energy division, now there is the political will the research will form an important part of the Community's biomass policy, which has been allocated research funds of 20 million ECU (European Currency Units) for a four-year period.

While farm ministers last week battled over price reductions to stem agricultural overproduction, particularly in cereals, there were signs of a consensus between research, industry and the farm lobby at a European Commission-sponsored meeting on biomass in Venice. According to Madron Seligmann, a British Member of the European Parliament, one hectare of land can produce four tonnes of ethanol and one tonne of biomass each year. The nine million hectares (of the Community's 150 million hectares of agricultural land) that grow surpluses could produce 32 million tonnes of ethanol every year. The 8 million tonnes of cereal surpluses alone represents 2 million tonnes of ethanol or 1.6 million tonnes of butanol-acetone.

The cost of getting rid of Community surpluses is estimated at 7,000 million ECU. According to Professor Andre Giraud (former French industry and energy minister and initiator of the French biomass and nuclear programmes), the Community would actually spend less by encouraging biomass production from surpluses than by subsidizing them, which could be done by a gradually declining system of incentives, and closer cooperation between agriculture and industry.

The need for a new European fuel blend policy has become more pressing as Europe prepares for lead-free petrol distribution in 1989 and stricter vehicle emission controls from 1988 as a result of the decisions by environment ministers on 20 March. Oxygenated products such as ethanol, methanol, acetone and butanol are already used to enhance the octane rating of some 60 per cent of fuel sold in West Germany, which conforms to the stricter Community standard of lead content of 0.15 grams per litre. These fuels may be suitable for the lean-burn engines due shortly. **Anna Lubinska**

Nuclear waste

Dump it here, please

THE British nuclear waste disposal agency, NIREX, is facing vocal opposition almost everywhere in Britain to its search for a new low and intermediate level disposal site. But its French equivalent, ANDRA, has had ten enquiries from the mayors of villages in central France inviting it to put its next site in their backyard.

The only problem is that although the French mayors are keen, for reasons of local development and employment, many of their people are not — for fear of the waste. Thus at one site, the village of Neuvy-le-roi near Tours on the Loire, the mayor was strongly in favour of ANDRA disposing low and intermediate level waste in the clay subsoil nearby, but his 1,000 villagers mounted a campaign against him. The mayor called a referendum: 63 per cent said no, and ANDRA has now dropped Neuvy-le-roi from its list.

However, still on the ANDRA list is Cholet, a town of 60,000 inhabitants south of Nantes and Angers, also near the Loire. Here the mayor is described as "very active" in attracting industry and employment, and the opposition is milder. "The behaviour at Cholet is very different from Neuvy-le-roi" said an ANDRA spokesman. The opponents there "are people you can argue with".

One of the arguments is money. The French government, unlike the British, offers local authorities concerned with potential nuclear sites both cash incentives and cut-price electricity. Thus Cholet stands to gain FF 30 million (£3 million) as a gift for housing and road improvements, and up to FF 1.5 million (£150,000) a year off local electricity bills, if ANDRA chooses it as a disposal site.

In Britain, however, the one named site — at Elstow near Bedford north of London — has met enormous opposition. Bedfordshire County Council has declared the area a "nuclear free zone" and written to the Secretary of State for the Environment, Patrick Jenkin, in strong terms, claiming Elstow "totally unsuitable as a site for a nuclear waste repository". Tempers rose further when the British government decided to allow NIREX to investigate the site by means of a "special development order" — a stratagem which avoids the need for a public inquiry.

The reaction to this British stick was perhaps predictable. The French style, offering a carrot, might have worked better, according to a study made for NIREX by Professor Terrence Lee of the University of Surrey. His survey figures show that 48 per cent of Britons would like monetary compensation (but paid to individuals rather than the council) for the building of a nuclear waste repository nearby, and a further 36 per cent would like compensation paid to the council for community benefit or some similar support — making a total of 84 per cent who say they could be bought off.

Robert Walgate.

Maritime museum plan scuttled

THE *Vityaz*, formerly the pride of the Soviet research fleet, which completed her last voyage in April 1979, is now anchored, a near-derelict with peeling paint and rotting woodwork, off Kaliningrad, *Pravda* reported recently. Plans to turn her into a floating museum have become bogged down in bureaucracy despite backing from ministerial and state bodies.

***Vityaz*, launched in April 1949, and the third Soviet vessel of her name (meaning "hero" or "champion"), spent most of her working life in the Pacific, carrying geological, ichthyological and meteorological expeditions. Her last, 65th voyage, however, ended with a triumphal tour of the major maritime states, including Italy, France, Spain, Portugal, the United Kingdom and Denmark, before she finally dropped anchor in Kaliningrad. It was originally hoped that the ship could go by canal to Moscow and a future as a museum. In the end, however, it was decided to leave her in Kaliningrad, the major fishing and commercial port of the Soviet Baltic coast. There was talk of a "Museum of the World Ocean" in the centre of the city, on the banks of the canal, with *Vityaz* as the prime exhibit.**

But then, in the words of *Pravda*, "it all turned out strangely". *Vityaz*, which had successfully weathered hundreds of thousands of miles of ocean voyaging, somehow became stuck fast when it came to completing the last few metres to her final berth. Now, almost six years after the *Vityaz* dropped anchor in Kaliningrad, only about half the money needed even for the minimum cosmetic refit is available in Kaliningrad. Money has, however, been spent on other things. A special harbour wall for the "memorial ship" has been erected, two smallish pre-war buildings have been renovated for the planned museum, and the surrounding terrain has been artistically laid out. According to the regional Communist Party secretaries, Yu Malakin and P. Kaz'min, public opinion in Kaliningrad still supports the idea of the ship museum, but someone has to make the official decision to build the museum complex. Once it is built, says Mr A. Shkurko, Deputy Minister of Culture of the Russian SFSR, his ministry will be delighted to take it over as a branch of the provincial museum of local history. No one, however, seems willing to assume responsibility for the decision to build.

Vera Rich

Indian science

Gandhi backs research

New Delhi

THE government headed by Rajiv Gandhi, who has pledged to prepare India for the twenty-first century, has allocated the equivalent in rupees of £900 million, or about six per cent of the government's budget for 1985-86, for scientific research and development.

The allocation for science represents only a marginal increase over that for the previous year but it is significant for its emphasis on research and development in non-conventional energy sources, biotechnology, high-power lasers and thermonuclear fusion.

As in earlier years, the lion's share of the budget goes to the Department of Atomic Energy (DAE), which is to set up a new centre for advanced technology at Indore, in Madhya Pradesh. Besides housing a new facility for plasma research, the centre will develop lasers for isotope separation and high power niodymium-glass lasers for laser-induced fusion. DAE also intends to set up a synchrotron radiation source, build superconducting magnets for its heavy-ion accelerator and establish a medical cyclotron for producing short-lived positron-emitting isotopes and radioactively labelled pharmaceutical preparations.

Budget allocations by sector (£ million)

Department of Atomic Energy	£210.0
Space	133.0
Industrial research	114.4
Defence research	104.0
Non-conventional energy	65.0
Agricultural research	55.5
Electronics	78.0
Oceanography	20.7
Meteorology	27.0
Other	93.0
Total	900.6

The Department of Ocean Development (DOD), which has assessed India's ocean thermal energy conversion (OTEC) potential at 50,000 MW, is planning to begin work this year on a 1-MW OTEC plant on the island of Lakshadweep. DOD will also place an order for an ice-breaker, estimated to cost £30 million, for undertaking expeditions to Antarctica. Previous expeditions were made in vessels chartered from Norway.

Electronics and biotechnology have been singled out as frontier areas. The government has abolished excise duty on computers and drastically reduced customs duty on their import. The Department of Electronics will launch this year a project to design a "fifth generation superminicomputer incorporating inference and knowledge based management functions". The Ministry of Science and Technology has earmarked a sum of £5 million for a biotechnology centre in New Delhi, its con-

tribution to the UNIDO plan for twin international centres for genetic engineering and biotechnology at Trieste, Italy and in New Delhi.

The Department of Environment has allocated funds for setting up a chain of botanical gardens for building a germplasm bank of economic plants, and seed and pollen banks for the conservation of threatened species. The department will also initiate this year a five-year £180-million programme to clean up the polluted river Ganges.

The Department of Space, which gets the second biggest share in the budget, plans to set up five regional remote sensing service centres to facilitate use of aircraft and satellite-sensed data and to provide trained manpower. Images received from Landsat will be augmented with data to be obtained from the French SPOT satellite. The space department will also start work on a new launch-pad for orbiting satellites in polar orbits. The polar launch station is expected to become operational in 1992.

K.S. Jayaraman

UK embryo research

Voluntary authority set up

THE British Medical Research Council (MRC), in association with the Royal College of Obstetricians and Gynaecologists (RCOG), last week replied to the government's inquiry into human fertilization and embryology (the Warnock report). MRC endorses the inquiry's recommendation for a licensing authority for research in these fields, and, recognizing that legislation will take some time, has set up a voluntary licensing authority under the chairmanship of Dame Mary Donaldson and consisting of MRC and RCOG scientists and doctors as well as lay members. The authority would both license experiments and draw up a code of practice. Although agreeing with the Warnock committee's 14-day limit on experimentation on embryos, MRC prefers that the limit be set in terms of stage of embryo development rather than days after fertilization, because of variations in the rates of growth between embryos.

MRC also seeks to address wider issues than those covered by the Warnock report: to define the term "embryo" (a viable conceptus developed from a fertilized egg and not tissue or cell cultures) and that "research" should include "new and untried treatment", a concept excluded in the Warnock report.

Human embryo research was not financially supported at all by MRC before the first British "test-tube" baby was born. Since then, MRC has recognized that research can improve understanding of infertility, may prevent some inherited diseases and congenital abnormalities and may produce safer contraceptive methods. Possible lines of research include:

- Finding the best culture conditions for egg maturation, of help to people suffering recurrent miscarriages after normal conception.
- Tests on defective sperm function, involving egg penetration and subsequent limited development of the fertilized egg. (Up to 50 per cent of human infertility is due partly to male-related disorders.)
- Some 60 per cent of eggs fertilized *in vivo* do not develop beyond implantation because of fetal abnormalities and unknown factors.
- Some congenital abnormalities (for example, Down's syndrome, spina bifida) probably occur during sperm and egg development.
- Better understanding of the development of chromosomal abnormalities.
- Investigation of infertility caused by production of antibodies to sperm in some women. Such research could lead to a contraceptive vaccine.

The MRC/RCOG report is one of many being submitted to the government. Eventually, legislation will result. But if the Powell bill (see *Nature* 21 February 1985, pp. 612 and 618) becomes law, considered reports such as the MRC statement will be so much wasted paper, for all research and development involving human embryos will be illegal. Techniques such as GIFT (gamete intrafallopian transfer), developed in Texas, where an unfertilized egg is removed from the ovary and immediately transferred to the fallopian tube where it can then be fertilized, would never be developed with such a bill operating. Later this month the first such baby will be born in Britain.

Maxine Clarke

Hazardous chemicals

Congress acts on clean air

Washington

IN the wake of the Bhopal disaster, pressure is mounting in the United States to enforce new limits on emissions of toxic substances from chemical plants.

The effort has been given a recent impetus by a series of revelations from Union Carbide (which operates a methyl isocyanate plant in West Virginia) and other chemical manufacturers concerning accidental and routine venting of hazardous substances. In responding to a survey conducted by the subcommittee on health and the environment under the chairmanship of Representative Henry Waxman (Democrat, California), 67 chemical companies acknowledged venting a total of some 204 chemical substances that the companies themselves identified as "hazardous". Many of the companies had been reluctant in the past to provide this data; Waxman approached the companies directly after discovering that the Environmental Protection Agency (EPA) had failed to collect this information in connection with its existing programme of regulating hazardous air pollutants.

The subcommittee is still analysing the data and will not release a complete report for several weeks. But a preliminary survey shows that at least a few plants emit surprisingly large quantities of hazardous substances. Borg Warner, for example, reported that one of its plants in West Virginia releases some 8,000 pounds of acrylonitrile, a known human carcinogen, into the air each day.

The subcommittee also found striking variations in emissions between similar plants run by different companies, and even between plants in different states run by the same company.

Under current EPA regulations, only five chemicals are controlled as hazardous air pollutants: vinyl chloride, benzene, asbestos, beryllium and mercury. Congress's environment committees have complained for years about EPA foot-dragging; EPA has listed 27 substances as "suspected hazards" but has not yet decided whether to issue control regulations. Although the legislative history of the 1970 clean air act shows that Congress expected EPA to consider regulating at least 12 specific substances (including chlorine gas, hydrochloric acid, several heavy metals, pesticides and radioactive compounds), the law itself gives the agency broad discretion to decide what constitutes a hazardous air pollutant and what action to take to limit release of pollutants into the atmosphere.

EPA has further provoked Congress by recently proposing a relaxation of controls on emissions of vinyl chloride, a known human carcinogen. Under existing rules, plants that produce vinyl chloride may only vent the chemical through relief valves under emergency conditions. Each time a venting occurs, EPA investigates the incident to determine if it could have been prevented; if it was preventable, the company is subject to civil or criminal penalties. Yet EPA records show that "emergency" venting of vinyl chloride is almost routine; one million pounds was released into the air during the period 1977-80. EPA is now proposing to recognize the fact by allowing each plant four to seven "free" ventings per year; EPA would no longer investigate each incident.

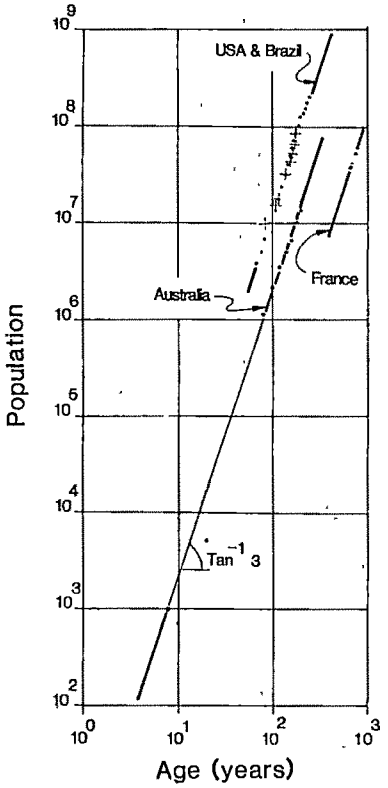
Waxman's subcommittee is working on a bill that would require EPA to produce regulations on a specific list of chemicals and with deadlines incorporated into the legislation. Similar efforts (albeit milder) have been passed by various subsets of Congress as part of proposed revisions of the clean air act, but always to die as the act itself founders on unresolved differences over other issues, notably acid rain controls. Waxman will try to have his bill considered as a separate piece of legislation, not tied to the clean air act.

Stephen Budiansky

Population growth

SIR — The literature, both popular and specialist, often refers to population growth rates as exponential. More often than not this implies merely a rapid rate of increase and not necessarily its proper meaning of a fixed proportional increase in a set timespan. For example, reference to almost any long-term demographic time series shows that the expansion is not truly exponential as it does not result in a straight line on a graph of the logarithm of population against time.

Now let us explore an alternative expansion rate — according to the cube of time since the modern nation was born. Australia is a nation for which the demographic record is known fairly precisely for the non-aboriginal population since its birth in the 1780s, about 200 years ago. There is thus very little flexibility to be tolerated on the birth date of the nation and retrospective analysis



leads to the initial date of 1780 from the application of this hypothesis to Australian population data[1]. In fact, that population generated from the "dumping" there a few years later of several hundred largely undesirable British citizens. The relation shown in the figure for Australia exhibits a general slope of gradient 3, that is, it represents an expansion according to the cube of time on this double logarithmic plot. The only anomalies are in the very early years when the embryonic settlement was subject to reduction by disease in the exotic environment and in the mid-nineteenth century when a gold rush generated an unnatural influx.

Encouraged by the well-documented Australian example, we might explore others. The figure shows similar relations for data from France[2], United States[3] and Brazil[4] from which it can be concluded that these nations began in the sense of a modern society in 1233, 1724 and 1783 respectively. Historians may care to comment on events that could justify that conclusion.

In the meantime, it seems appropriate to draw attention to the far more accurate demographic projections available from this model than from the variably time-dependent exponential expansion models commonly found in the literature. The reader can test this conclusion by exploring, say, the 50-year forecasts that would have been made at decadal intervals over the past century or so using both this cubic expansion and any exponential model. The results are most encouraging and promise much for demographic planning.

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1 Cameron, R J *Official Yearbook of Australia* Vol 61, 135 (Australian Bureau of Statistics, Canberra, 1976)
2 *Annuaire Statistique de la France* Vol 88, 25 (Institut National de la Statistique et des Etudes Economiques, Paris, 1983)
3 Teir, J E *et al Statistical Abstracts of the United States* Vol 100, 6 (US Government Printing Office, Washington DC, 1979)
4 *Anuario Estatistico do Brasil*, 39 (Instituto Brasileiro de Estatistica, Rio de Janeiro, 1971)

Viscosity, noses and IQ

SIR — P.M. Gaylarde's assertion (*Nature* **313**, 425; 1985) that I speculated "that the shape of a person's nose determines his IQ" is both offensive and inaccurate. In fact, what Gaylarde interpreted as an "attempt to establish spurious grounds for white supremacy" in a manner "as unsavoury as those...used to justify the extermination of Jews and Gypsies in Hitler's Germany", was, both in intent and content, exactly the opposite. I urge readers who saw only Gaylarde's response to read what I wrote before passing judgement (*Nature* **311**, 515; 1984).

It is true, as Gaylarde states, that "the viscosity of moist air is less than that of dry air", but the relevant comparison is between the cool dry air of temperate zones and the hot humid air of the tropics. Unlike liquids, gases gain viscosity with increases in temperature, and that gain more than offsets the reduction from increased humidity. For example, air going from 0 to 40°C and simultaneously from dry to saturated (maximizing the effect of humidity), gains about 20 micropoises of viscosity as a result of increased temperature[1] while it loses about two micropoises of viscosity from increased humidity[2] — a net gain of 18. Gaylarde is also wrong to think that nose shape, length and hairiness have little effect on airway resistance[3]. If the length of a tube is doubled, pressure must be increased twofold to maintain constant flow, and if the radius of a tube is halved, pressure must be increased an additional sixteenfold to maintain flow — a 32-fold increase. Add the fact that saturated hot air (say 40°C) contains less oxygen (about 7 per cent less), and one can begin to appreciate the value of a short wide hairless nose in hot humid climates.

But the brunt of my nose argument, the entirety of which was not essential to my point, was that it might be disadvantageous not to have a dust-trapping nose in dusty environments. In support of this speculation I pointed out that "among American racial and ethnic groups, only blacks and non-Japanese orientals have a conspicuous prevalence of lung disease". I agree with Gaylarde that lung disease "is related to socioeconomic factors", but its relative inconspicuousness in hispanics leaves my point intact.

The objective of my contribution was to explain the fact that heritable differences in IQ need not be attributed to differences in "IQ genes", and I have argued that any heritable difference in IQ between races is especially unlikely to be caused by such genes[4]. My point was that adaptations taken out of their evolutionary context (such as pale thin skin and long, thin, hairy noses in the tropics) are liable to be disadvantageous and could have an incidental effect on IQ. I pleaded that such questions should be investigated because "there are many factors that something could be done about if we knew what they were", for example, "people with non-dust-trapping noses can have humidifiers in their homes during the winter and take care to wear a dust-mask when doing a dusty job". And I lamented the fact that "looking for genetic differences that incidentally affect IQ is politically unacceptable". Gaylarde has proven me right in this last regard.

Misrepresentation of facts, misreading, an astounding failure to comprehend and knee-jerk reply aside, as the son and nephew of men who fought against Hitler's Germany, and as one who grew up in a home where racists, anti-semites and fascists were clearly "the enemy", I resent Gaylarde's ludicrous insinuations.

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1 Weast, R C *Handbook of Chemistry and Physics* F-39 (CRC, Cleveland, 1965)
2 Kestin, J & Whitelaw, J H *Humidity and Moisture* Vol III, 301 (Reinhold, New York, 1965)
3 Negus, Z *The comparative anatomy and physiology of the nose and perinasal sinuses* (Livingstone, London, 1958)
4. Hartung, J *Curr Anthropol* **21**, 131-132 (1980)

The importance of impatience

Unifying gravity with the other three fundamental forces has been brought a large step nearer. But there is a long way still to go.

IN a week in which there appears in *Nature* an account of a theoretical framework that may be the best hope yet that theories of particle physics will be united with gravitation, a reminder of how frustrating this problem has become may be in order. The point is nicely illustrated by Dr C. Villi of the University of Padua in the latest issue of *Il Nuevo Cimento* (85A, 175; 1985).

Villi's interest is simple, to explore the question whether the mathematical infinities that have plagued the calculation of particle properties, and which persist, are merely consequences of leaving gravitation out of the calculations. For, as he puts it, "the most significant aspect of the divergences encountered in quantum field theory is that they do not exist in Nature". His method is straightforward: to suppose that true curved space-time is everywhere approximated by flat Minkowski space-time, essentially that of special relativity, from which it follows that the function which defines the approximation at each point of space-time (the form factor) must satisfy Einstein's equation of gravitation.

At this point, another of Villi's discontents comes to the surface. Soon after Einstein first formulated his equations of gravitation (in 1917), he confused the issue by adding to the simplest form admissible on the grounds of geometrical consistency an extra term containing a constant, the gravitational constant. Equations with this extra term are consistent with the physical arguments leading to general relativity, while the presence of the constant made it possible to construct a finite universe containing a uniform distribution of matter whose density was not identically zero.

Villi is scornful of the way the cosmological constant has been dealt with ever since. It is agreed that it must be numerically small, but is it right to follow Pauli (and Einstein's second thoughts) in dropping it altogether? Villi is in no doubt "No efforts have been made to understand the *raison d'etre* [of the cosmological constant] This is indeed astonishing because its cosmological significance is purely accidental... the parameter would appear in Einstein's equations even if cosmology had never been thought of."

Villi's objective is to show that the cosmological constant can somehow smooth out (with the help of his form factor) the infinities that arise in the calculation of particle properties. Although accepting that the constant is numerically small for large-scale phenomena, he would have it very large on the scale of particle phenomena.

There are precedents for this line of thinking. One way of making a connection, however tenuous, between gravitational and particle phenomena is to remark that the bending of distant starlight at the limb of the Sun (one of the three accepted tests of general relativity) could otherwise be accounted for if the refractive index of the vacuum were different from unity, which may in turn be explained if the vacuum is polarizable. Villi notes that all attempts to forge such connections have been defeated by objections of doubtful weight.

"In surveying this matter, one is oppressed not only by objective difficulties, but by arguments open to serious doubts and by unnatural assumptions which breed confusion and scepticism. Too many dissertations based on unfruitful and controversial theoretical subtleties have contributed to shattering the problem of the role played by gravity in the subnuclear universe into fragmentary aspects generally contradicting each other . we shall pursue a different line of thought . to overcome the apparent irreconcilability of general relativity with quantum field theory, a dilemma that may provide the seed for a radical rethinking of some physical concepts and of the theoretical schemes developed."

Fighting talk? Villi confesses that he cannot at this stage provide the general prescription that his programme requires, but goes on to give some telling if particular examples. The form-factor way of regarding space-time as locally flat does get rid of some of the infinities arising in solutions of the Kelin-Gordon equation, for practical purposes the generalization of the wave equation for photons to particles with non-zero mass and spin. He thinks it important to tackle the problem in the way in which Feynman first made quantum electrodynamics respectable, by the "path-integral" method, but only after this statement:

"I confess my inability of profiting heuristically from the jungle of theoretical approaches invented in order to transpose to general relativity Feynman's methodology It is difficult to see a way out of such a puzzle, not only because there are conceptual black corners still unexplored, but also because one does not know how to evaluate the formulae thus obtained, nor how to give them a mathematically precise meaning " And, having translated Feynman into general relativity which, Villi warns, "is not very exciting", he has this stirring comment on the way in which field theorists get rid of their infinities.

The "daring manipulation of infinite quantities based on the killing of infinities by counter-infinities raises questions which indicate the need for a thorough revision of the renormalization theory . there remain inherent mathematical ambiguities in handling divergent expressions it is a challenging task to find a way towards an alternative formulation of quantum electrodynamics, free from the slaveries of renormalization "

Where does it all lead? Villi is in no doubt. The vacuum fluctuations beloved of particle physicists must endow empty space with what Einstein called an energy-momentum tensor that differs from zero, and the consequence must be a short-range "cosmological constant" that not merely differs from zero but is very large. And "this has nothing to do" with the large-scale cosmological constant related to the mass-density of the universe at large. Villi's other important general precept is that it may be an illusion on the part of the field theorists to look for a unification on equal terms of the four fundamental forces (strong and weak nuclear interactions, electromagnetism and gravity). Perhaps, he says, gravity always enters on a qualitatively different footing, and should be handled differently from the start.

What M.B. Green (see p.409) has to say notwithstanding, Villi should not be scorned There is a natural temptation among those who construct theories to account for phenomena not previously well categorized to suppose that only one mathematical framework will accommodate the phenomena, and the trick is to find it. That is how it is at first, but time accretes other ways of solving these problems.

It is too soon for Green to have figured out whether what he has done (and what earlier users of Kaluza-Klein theories, in which redundant "internal" dimensions are collapsed onto ordinary space-time, have attempted) fulfils the objective of Einstein and his later collaborators (Infeld and Bohm, for example) to define the "hidden variables" that would make quantum mechanics determinate. For the time being, it is more important to win some physics from the mathematics. But Villi and his like will continue to be angry if they are forever fobbed off with the answer that their questions cannot be answered in the terms in which they are put. **John Maddox**

Halley's comet

Prospects for Giotto and Halley

from M.K. Wallis

AT the final pre-launch preview*, scientists involved in the Giotto mission to Halley's comet concluded that the risk to the spacecraft from dust particle impacts (see *Nature* **303**, 659; 1983 and **305**, 756; 1983; *ESA J.* **7**, 15; 1983; *Earth, Moon Planets* **30**, 31; 1984) is considerably less than had been feared.

Initial pessimism had centred on the likelihood of impacts that would cause a deviation of Giotto's spin axis in excess of 1°, when radio contact would be lost. Although milligramme and smaller-sized grains impacting the protective shield will contribute to the deviation, it is now agreed that the original pessimism was based on an over-weighting of the rather improbable grains exceeding 0.1 g, which might anyway smash through the rear shield and destroy instrumentation.

Predictions of survival are based on predictions of the density and size-distribution of dust particles. The probability of survival against 1° perturbations is now given as around 90 per cent on the nominal dust model for a 500 km flyby distance, although calculations need extending to give a secure guide for future targeting decisions.

Much scientific effort has gone into elaborating models of the comet's dust coma; rather than assuming instantaneous formation, it is now agreed that dust grains can reside in the coma for weeks or even months, forming into anisotropic structures purely because of the comet's motion around the Sun. Model calculations at the NASA-Jet Propulsion Laboratory (JPL) have been extended to encompass long-lived dust grains and are now in agreement with an alternative analysis by ESOC scientists (Fertig, J. & Schwehm, G.H. *ESA Bull.* **38**, 36; 1984).

There has been concern about much higher dust densities in jet-like regions. JPL scientists (Sekanina, Z. & Larson, S.M., *Astr. J.* **89**, 1408; 1984) have applied digital image-enhancement techniques to photographs of comet Halley's 1910 apparition, and infer enhanced densities, some 10-100 times the mean. It is now accepted, however, that these would be of submicron size (like cigarette smoke) and present a desirable target rather than a threat to the spacecraft's integrity. Study of the jet phenomenon might, alternatively, indicate where to expect the dangerous, slowly-moving, millimetre-sized grains.

Originally, the mission was scheduled to terminate one hour after closest approach, supposing that the spacecraft is unlikely to survive. But there was pressure from experimenters at the meeting to retain transmission options for two days on the outward flight through the extensive gas coma.

The meeting heard that the Pathfinder concept agreed with the Soviet Space Agency is going ahead; data from the Soviet VEGA spacecraft will be used to cut down the error in targeting. If the two VEGAs are tracked by very long baseline radio-interferometry, the error should be under 100 km. Supplementary ground-based astrometry is also to be investigated.

The reserve of one month in the original schedule has now been used up, with final instrument acceptance checks due in late March. No significant problems have arisen on subsystems or experiments during the system test programme, although the spacecraft as a whole is hotter than predicted, requiring modifications to radiating surfaces. Assembly and final testing of the television camera is running late, so installation and checking procedures have been reorganized around it. One advantageous change to the original schedule gives scientists the option of integrating fully-calibrated versions of their experiments just before delivery of the spacecraft.

The planned launch campaign is due to commence at the beginning of May 1985 with an expected launch date of 2 July 1985 on an Ariane 1 vehicle from the European launch site at Kourou, French Guiana. The optimistic hopes of securing data on the inner coma and pictures of the nucleus from a few hundred kilometres seem likely to be achieved on the basis of this review. □

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*European Space Research and Technology Centre, Holland, 19-20 Feburary, 1985

Chemical evolution

Origin of biomolecular chirality

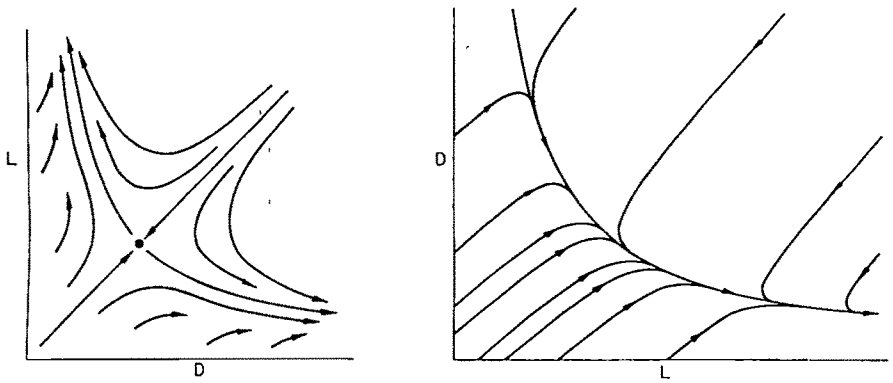
from Stephen Mason

THEORIES accounting for the ubiquity of the L-amino acids and the D-sugars in the biochemistry of living organisms centre on mechanisms for the selection of enantiomers of one hand from the heterochiral mixture of a racemic substrate, and for the propagation of optically pure homochiral products. Surprised to learn, in 1953, that the origin of biomolecular handedness still appeared to be problematic, Frank proposed a homogeneous chemical kinetic mechanism for an open flow-reactor system[1]. The mechanism contained no internal bias, and the dominance of the L-amino acids and the D-sugars in the natural products, rather than their respective enantiomers, seemed to be wholly a matter of chance. By extending the mechanism and incorporating a recently evaluated internal bias, Kondepudi and Nelson[2] now define the particular conditions under which a small electroweak advantage factor for the L-series of amino acids and polypeptides[3] leads to a determinate choice in the evolution of homochiral biomolecules from an achiral or racemic substrate.

Frank's model avoided the direct intervention of external agencies, such as a chiral field or panspermatic celestial seeding, and the various heterogeneous mechanisms advocated in the grand debate on the subject in the correspondence columns of *Nature* during 1898 following F.R. Japp's paper on "Stereochemistry and Vitalism"[4]. Ironically, that correspondence ignored the 1884 conjecture of Pasteur that a dissymetric force pervades the physical world, extending even to the crystallization dish used for the separation of enantiomers. The discovery, in 1956, of the non-conservation of mirror-image symmetry in the weak nuclear interaction supported Pasteur's surmise, and the subsequent unification with electromagnetism has allowed the characterization of both the sign and the magnitude of the universal chiral electroweak interaction. The sign is in accord with the observed products of natural selection. The L-amino acids and the L-polypeptides are more stable than their D-enantiomers. But the advantage ratio of the electroweak enantiomeric energy difference (ΔE_{ew}) to the thermal energy (kT) at ambient temperature is only about 10^{-17} (ref.3), too small according to one school of thought[5] to affect the outcome of biochemical evolution.

Frank envisaged an open reactor system with a continuous input of an achiral substrate from which both enantiomers, L and D, are formed (see figure). Each of the optical isomers autocatalyses its own production and competitively inhibits propagation of its enantiomer. In general, the dynamic generation of a racemic product is metastable and subject to a bifurcation catastrophe. Chance fluctuations sooner or later trigger a kinetic switch to one of the homochiral reaction channels, producing either the L-isomer or the D-enantiomer. Which is produced, in the absence of an internal bias or an external perturbation, depends on the sign of the chance fluctuation[1].

The general solution to the kinetic relationships of the extended mechanism is shown to be a cubic equation in the enantiomeric excess of the handed products, $(\alpha/\beta = ([L] - [D])/([L] + [D])$, where [L]



The time evolution of L and D enantiomers from an achiral or racemic substrate in an open flow-reactor system, with an autocatalytic production of each isomer and an enantiomeric cross-inhibition: left, for identical values of the activation parameters of corresponding enantiomeric intermediates and right, for a small bias favouring the production of the L-isomer. Metastable racemic production is represented by the unique point in the left hand graph, to which either an achiral or a racemic substrate leads, and from which the two homochiral production branches diverge. Modified from reference 1.

and [D] refer to the concentrations of the enantiomers[2]. One root of the cubic equation, correlating with a small input concentration of the substrate into the flow-reactor system, corresponds to a vanishing enantiomeric excess, $(\alpha/\beta) = 0$, giving a racemic product. The other two roots ($\alpha/\beta = +1$ or -1) correlate with high substrate input concentrations, and correspond to the two homochiral reaction branches, giving only the L-isomer or the D-enantiomer. At the transition point, where the racemic production process becomes metastable and bifurcates into the oppositely handed reaction channels, the system develops a magnified hypersensitivity to minor inequalities between the activation parameters of corresponding enantiomeric intermediates in the two homochiral reaction branches. Thermal fluctuations at the temperature, T, of the system randomize and annul any initial trend towards the D or the L production branch unless the difference in activation energy, ΔE, between the enantiomeric intermediates satisfies the relationship $(\Delta E/kT) > 10^{-17}$ (ref.2).

At ambient temperature, the electroweak advantage ratio for the L-series of amino acids and polypeptides, $(\Delta E_{ew}/kT)$, meets the lower limit of the relationship, so that selection of the reaction branch for the L-series becomes determinate over a limited range of conditions. With realistic rate constants for the elementary kinetic stages of the model mechanism, the homochiral reaction branch for the L-series is selected with a 98 per cent probability if the passage through the critical hypersensitive transitional bifurcation state is slow. Over 10^4 years this would correspond to an increase in the input concentration of the substrate by 10^{-2} to 10^{-3} moles in a flow reactor with the volume of a small lake, approximately one kilometre in diameter and some four metres deep. This estimate takes into account quantifiable disordering effects, such as the known rates of spontaneous racemization of the amino-acid enantiomers, and the minor enantionmeric photodiscrimination of solar radiation at twilight, when there is a small net circular polarization, oppositely handed at dawn and dusk[2]. □

1 Frank, F C *Biochim biophys Acta* 11, 459 (1953)
2 Kondepudi, D K & Nelson, G W *Nature* 313, 438 (1985)
3 Mason, S F & Tranter, G E *Proc R Soc* A397, 45 (1985)
4 Japp, F R *Nature* 58, 452 (1898)
5 Morozov, L L, Kuzmin, V V & Gol'danskii, V I *Sov Sci Rev D physiochem Biol* 5, 357 (1984)

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Cell biology

Baseless flagellation

from Jeremy S. Hyams

COMPARED with its enigmatic alter ego, the centriole, the basal body is a fairly straightforward organelle. Basal bodies sit, appropriately, on the base of eukaryotic cilia and flagella. Unlike the structures associated with the base of bacterial flagella, they are not actively involved in motility. Rather, they provide the template for the assembly of the 9+2 axoneme and, through their association with various rootless structures, anchor the beating cilium or flagellum against the generated torque. This rather comfortable view of basal body function may, however, have to be re-examined in view of a paper by H.J. Hoops and G.B. Witman on the green alga *Chlorogonium*[1].

In design, the flagellar apparatus of *Chlorogonium* resembles that of its better studied relative *Chlamydomonas*[2]. The two flagella arise from the anterior of the cell in a 'V' configuration. The basal bodies which from the crotch of the V are maintained by a group of striated fibres and serve as the focus for a series of microtubule rootlets that head off towards the cell's posterior. During forward swimming the two flagella sweep away from each other in a motion which, superficially at least, resembles a human swimmer's breast stroke. Unlike *Chlamydomonas*, where the flagella are resorbed before mitosis, *Chlorogonium* contrives to swim during cell division, the parental flagella remaining attached to the anterior-most daughter cell. Electron microscopy of these dividing cells reveals that the basal-body complex detaches from the flagella to become associated with the poles of the spindle.

Remarkably, not only do the flagella continue to beat in the complete absence of their basal apparatus but the cells still exhibit their normal photophobic and phototactic responses. In *Chlamydomonas*, these responses involve distinct changes in the pattern of flagella beating. During the photophobic response the flagella switch from the asymmetrical ciliary mode used during forward swimming to a symmetrical flagellar waveform that drives the cells backwards[3]. Phototaxis, on the other hand, involves the inactivation of one flagellum whilst its partner continues to beat, causing the cell to turn[4].

Experiments on detergent extracts of either isolated flagella or whole cells have shown that both responses are mediated by calcium ions[4-6]. A rise above 10^{-6} M in the intracellular concentration of calcium triggers the photophobic reaction[6,7], whilst a differential response of the two flagella to submicromolar levels of calcium probably underlies phototaxis. The two flagella of *Chlamydomonas* can be distinguished on the basis of their position relative to the asymmetrically positioned eyespot. The flagellum furthest from the eyespot is inactivated below 10^{-8} M calcium whilst the other flagellum continues to beat. Conversely, the flagellum closest to the eyespot is inactivated by 10^{-7} - 10^{-6} M calcium whilst the other flagellum remains active.

By obtaining similar results with dividing *Chlorogonium* cells, Hoops and Witman show that the complex behavioural responses of green algae are intrinsic to the flagella themselves and independent of accessory structures associated with the flagellar base. Taken together with the recent identification of a rhodopsin in *Chlamydomonas*[8,9], these new findings suggest that our understanding of tactic responses of eukaryotic microorganisms may soon approach the sophistication of equivalent studies on prokaryotes. □

1 Hoops, H J & Witman, G B *J Cell Biol* 100, 297 (1985)
2 Ringo, D L *J Cell Biol* 33, 543 (1967)
3 Boscor, J S & Feinleiv, M E *Photochem Photobiol* 30, 499 (1979)
4 Kamiya, R & Witman, G B *J Cell Biol* 98, 97 (1984)
5 Bessen, M, Fay, R B & Witman, G B *J Cell Biol* 86, 446 (1980)
6 Hyams, J S & Borisy, G G *J Cell Sci* 33, 235 (1978)
7 Schmidt, J A & Eckert, R *Nature* 262, 713 (1976)
8 Foster, K W *et al Nature* 311, 756 (1984)
9 Berg, H C *Nature News and Views* 311, 702 (1984)

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Astronomy

Direct estimate of Hubble's constant from the double quasar

from C. Martin Gaskell

DETERMINATION of the time delay between observed variations in the two images of the first gravitationally lensed quasar to be discovered (0957+561 or 'the double quasar') has enabled Hubble's constant (H_0) to be estimated directly[1]. This way of measuring H_0 short-circuits the many intermediate steps in 'classical' estimations but, unfortunately, is very model dependent.

The double quasar was discovered by D. Walsh, R.F. Carswell and R.J. Weymann as a pair of quasars, 6 arcseconds apart, with virtually identical spectra[2]. Subsequent research verified their initial guess that these two images come from one and the same quasar: the gravity of a galaxy in the line of observation is bending the light and producing two images of the more distant quasar[3]. Long before this system was discovered it had been pointed out that variability of a gravitationally lensed object would allow an estimate of the distances involved and hence of H_0, the constant that relates the velocity of recession of the galaxy or quasar to its distance ($1/H_0$ is the approximate age of the Universe)[4]. In essence, the method is simple: if the shape of the various light paths is known, then the time delay due to the difference in light travel time) between seeing a given variation in one image and the other, gives the scale size. (For the double quasar there are actually three images, but the third is very faint.)

Like virtually all quasars[5], 0957+561 was quickly shown to exhibit 'flickerings' in brightness (of 30 per cent or so) that last weeks or months[6]. Once that was known, a number of observatories mounted photometric monitoring programmes, and, while waiting for the results, theoreticians refined the model of the mass distribution in the 'lensing' galaxy and the cluster in which it is located[7,8]. The expectation was that variability of the northern image would precede variability of the southern image by a couple of years.

R. Florentin-Nielsen and K. Augustesen of Copenhagen University Observatory were among the observers following 0957+561. They took repeated photographs with a 20-inch Schmidt camera, a small telescope by modern standards, and in much poorer sky conditions than United States' observers, but with the advantage of being far enough north to follow the double quasar all year round. In early 1980, the southern image brightened while the northern one stayed constant; the southern image was presumably showing some event that could have been seen a year or two earlier in the northern image. Both images then stayed constant in brightness until 1982 when the northern image started to brighten by 30 per cent while the southern image stayed constant. In 1983, the northern image started to fade. There had been a clear 'event'. It was then just a matter of waiting until the southern image followed suit for an estimate of H_0 to be made. By July 1984 it was clear to Florentin-Nielsen that the long-awaited rise in the southern image had begun. In his recent paper[1], he estimates the time delay to be 1.55 ± 0.1 years and derives H_0 as 77 km s^{-1} Mpc^{-1} corresponding to a Hubble age ($1/H_0$) of 13 x 10^9 years.

How reliable is this estimate of H_0? The formal observational error is only ± 5 km s^{-1} Mpc^{-1}. Classical astronomical methods (for example, using Cepheid variable stars) currently yield H_0 values of 43–95 km s^{-1} Mpc^{-1}, and a recent review favours 68 or 82 km s^{-1} Mpc^{-1}, depending on the methods used. The latest work on type I supernovae[10] favours a value of 58 km s^{-1} Mpc^{-1}. The Florentin-Neilsen value is clearly well within the range under debate.

Within a year or two the observational uncertainty in the time lag should have been narrowed down by a factor of two or more. But the real uncertainty is greater. To calculate H_0, Florentin-Nielsen relies on detailed modelling by Borgeest and Refsdal[8] of the mass distribution in the lensing galaxy and the cluster that contains it. The question is whether Borgeest and Refsdal have correctly modelled the influence of the cluster on the estimate of H_0. They believe it is less influenced by the cluster parameters than had been feared by, for example, Dyer and Roeder[7], and that the effect of the cluster galaxies might be leading to a slight overestimate of H_0 from the double quasar. Nevertheless, it remains the largest source of error.

Fortunately, the free parameters in the mass distributions may soon be narrowed down by radio observations of very high spatial resolution that are underway. The models of the mass distributions will have to match the twisting and distortion seen in the radio structure. If they do, then there is some confidence that the time delays, and hence H_0, have been calculated correctly. Meanwhile, optical observers will be calmly continuing to follow the two images to ensure that the current outburst in the southern image really matches the 1982 – 3 record for the northern image. If not, some major rethinking will be necessary. □

1 Florentin-Nielsen, R *Astr Astrophys* **138**, L19 (1984)
2 Walsh, D, Carswell, R F & Weymann, R J *Nature* **279** 381 (1979)
3 Young, P J, Gunn, J E, Kristian, J, Oke, J B & Westphal, J A *Astrophys J* **241**, 507 (1980)
4 Refsdal, S *Mon Not R astr Soc* **128**, 307 (1964)
5 Angione, R J & Smith, H J in *IAU Symposium No 44 External Galaxies and Quasi-Stellar Objects* 171 (Dordrecht Reidel 1972)
6 Keel, W C *Astrophys J* **255**, 20 (1982)
7 Dyer, C C & Roeder, R C *Astrophys J* **241**, L133 (1980)
8 Borgeest, U & Refsdal, S *Astr Astrophys* **141**, 318 (1984)
9 van den Bergh, S *Q J R astr Soc* **25**, 137 (1984)
10 Arnett, W D., Branch, D & Wheeler, J C *Nature*, **314**, 337 (1985)

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Parasitology

Towards schistosomiasis vaccines

from F.E.G. Cox

SCHISTOSOMIASIS, one of the world's major diseases, is caused by adult worms that inhabit blood vessels associated with the lower intestine or bladder. The worms survive in these immunologically exposed sites largely because while migrating there as juveniles they acquire host-like surface antigens from the skin, where they enter the body. So effective is this disguise that it is not at all clear if there is any effective immunity in man [1]. Vaccines consisting of irradiated juvenile worms[2] have been the basis of successful field trials in cattle in the Sudan [3] and China[4] but are unlikely to be acceptable for human use. Therefore, several groups have investigated the potential of extracts of juvenile and adult worms. The results are disappointing, although some purified surface antigens have been used with limited success in mice and rats. The most recent study indicates that some protection can be induced in monkeys[5]. This is a major step forward.

Maire Smith and John Clegg, of the Wellcome Research Laboratories, have identified and purified two surface antigens associated with both juvenile and adult worms [5]. The larger molecule, given twice with alhydrogel adjuvant, induces partial protection both in mice and cynomolgus monkeys. The smaller antigen, which could be extracted from the adult worms only with difficulty, also protects mice. These results are promising in that antigens common to the surfaces of both juvenile and adult worms have some protective capacity, but disappointing in that the levels of protection are low and inconsistent. Nevertheless, they indicate that the approach of identifying and purifying surface antigens is worth pursuing. The next step is to clone the genes encoding these molecules, to express them and to test the products.

Although it is now possible to predict future directions of research, it would be

foolish to assume that all is now plain sailing. André Capron and his colleagues in Lille have shown that an IgG2a antibody is involved as a bridge between a schistosome surface antigen and the eosinophils that act as killer cells in the protection of rats against schistosomiasis[6]. More recently, however, they have shown that the same antigen can also elicit the production of an IgG2c antibody, which actually blocks the protective activity of the IgG2a[7]. In developing a vaccine based on purified antigens, therefore, it will be essential to ensure that the antibodies produced do not actually block some unrecognized protective mechanism. Similarly, care will have to be taken to ensure that a vaccine does not result in the death of the migrating juveniles in an unsuitable part of the body. In rats immunized with irradiated juveniles, the worms in the vaccine die in the skin or lungs, whereas those of a challenge infection die during migration from the lungs to the liver[8]. The dangers of the release of antigen in the lungs or liver are obvious, placing yet another constraint on the development of a vaccine.

The relative lack of success in vaccinating animals, especially primates, against schistosomiasis has led some workers to investigate less conventional methods, including the induction of non-specific immunity by the use of immunostimulants. Results obtained in baboons have been equivocal but do suggest that non-specific activators of macrophages and monocytes can provide some protection against schistosomiasis[9]. Overall, specific and non-specific immunization can at best only reduce the worm burden in the host to about half, which may be of advantage to the individual but not to the community, as it would have a negligible effect on transmission.

Some may argue that a vaccine against schistosomiasis is not really necessary because excellent drugs are available. But the first warnings have already been sounded that this situation may not last for long. Praziquantal is an extremely effective and safe drug, albeit somewhat expensive, but several serious side effects have been reported recently from a small community in Zaire[10] and it must be assumed that similar reports will be forthcoming from elsewhere. Both chemotherapy and vaccination have advantages and disadvantages. Ideally, a programme of schistosomiasis control would incorporate the best of both approaches, with health education and the use of molluscicides. □

1. Butterworth, A.E. *et al. Immun. Rev.* **61**, 5 (1982).
2. Bickle, Q.D. *et al. Parasite Immun.* **5**, 499 (1983).
3. Majid, A.A. *et al. Am. J. trop. Med. Hyg.* **29**, 452 (1980).
4. Hsu, S.Y. *et al. Am. J. trop. Med. Hyg.* **32**, 367 (1983).
5. Smith, M.A. & Clegg, J.A. *Science* **227**, 535 (1985).
6. Grzych, J.M. *et al. J. Immun.* **129**, 2739 (1982).
7. Grzych, J.M. *et al. J. Immun.* **133**, 998 (1984).
8. Ford, M.J. *et al. Parasitology* **89**, 461 (1984).
9. Sturrock, R.F. *et al. Parasitology* **90**, 101 (1985).
10. Polderman, A.M. *Trans. R. Soc. trop. Med. Hyg.* **78**, 752 (1984).

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Palaeontology

Outlines of the distant past

from Peter Gambles

THE dawn of life was also the dawn of death. Since the remote Precambrian, more than 3,500 million years ago, countless plants and animals have died, but only an incredibly small fraction have been preserved in the rocks as fossils. Yet it is this meagre record that charts the path of evolution.

Most people will be familiar with the more common forms of preservation, such as sea-shells in limestones or the great mineralized skeletons of dinosaurs in museums. Although palaeontologists can glean a lot of information from the morphology of these 'hard parts' of animals and from their tracks and marks (known as trace fossils), ideas concerning their appearance and behaviour when alive are difficult to substantiate. But every once in a geological while, circumstances combine to yield fossils with exquisitely preserved outlines of 'soft parts' — limbs and internal organs. Extraordinary fossil biotas of that type were the subject of a recent meeting at the Royal Society*.

Many of the specimens recovered are objects of (sometimes morbid) interest in their own right. The structure of the leg of a trilobite from the Burgess Shale (Fig. 1), which died 530 Myr ago, or the outlines of the feathers of *Archaeopteryx*, help relate these creatures to their modern counter parts. Other finds are so outlandish they can barely be classified (indeed, one has been called *Hallucigenia*).

Trace fossils of crawling animals, such as horse-shoe crabs, are not uncommon. But in the Solenhofen limestone (150 Myr) a dead animal is found at the end of the tracks. Elsewhere there are insects trapped in amber, mammoths in ice, even a mother ichthyosaur dead in childbirth. It seems that an essential requirement for this exceptional preservation is that the environment suddenly becomes inimical to life. This can happen when there is rapid deposition of a smothering blanket of mud on otherwise normal seafloors, or if creatures fall or are swept into an anoxic trap. The Burgess shale formed in such a trap at the base of a Cambrian reef.

Perhaps of even greater significance is the preservation of wholly soft-bodied organisms. About 70 per cent of modern biota is soft-bodied and S. Conway Morris (Cambridge University) asked whether this proportion has varied with time. Effectively no shells or exoskeletons have been preserved from before the so-called Cambrian evolutionary explosion. But detailed studies of the Cambrian Burgess Shale fauna suggest that there has been little change in the proportion of soft-bodied biota over the past 600 Myr. Put another way, more than two thirds of the original biota are not represented in most 'good' fossil localities, so any fossils of soft-bodies organisms may help fill large gaps in our understanding of the ecology of ancient environments. As an example, there has seemed to be a lack of predators in 'typical' shelly Cambrian faunas, which has led some workers to believe that the world was then a tamer place. But, according to Conway Morris, several soft-bodied organisms from the Shale look decidedly ferocious; their preserved gut contents identify them as predators.

*'Extraordinary fossil biotas: their ecological and evolutionary significance', Royal Society, London, 20-21 February 1985.

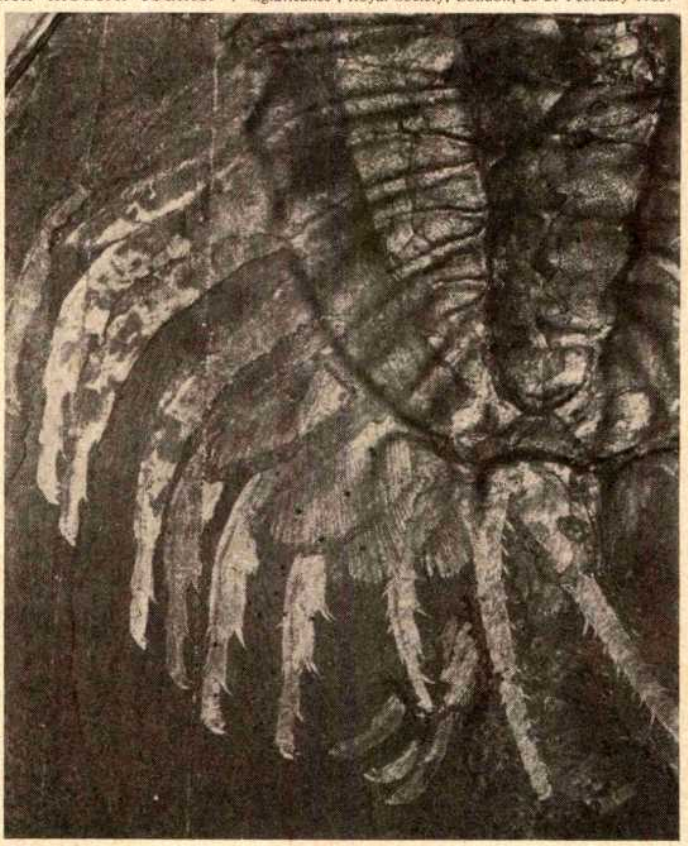


Fig. 1 The trilobite *Olenoides serratus* from the Burgess Shale, showing details of the legs and gills (H.B. Whittington, Cambridge University).

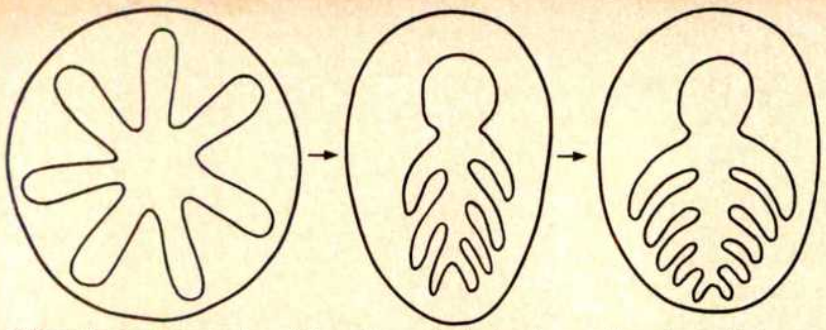


Fig. 2 Evolutionary progression of the metazoan bodyplan from odd-number radial symmetry through "glide reflection" to true bilateral symmetry (M. Fedonkin). See text for details.

A question that arises out of this reappraisal concerns the role of soft-bodied organisms in the evolutionary radiation of the early Cambrian. Many of the more enigmatic fossils seem to represent body plans that are not exhibited by any living creature. The present-day Metazoa (multicellular animals) are dominated by bilaterally symmetrical body plans, often with a degree of segmentation. The coelenterates, with concentric or radial symmetry, today constitute only a small part of the animal kingdom. But M. Fedonkin (USSR Academy of Sciences, Moscow), studying the soft-bodied biota from the Vendian (about 650 Myr) of the White Sea Russian Platform and polar Siberia, concludes that ≥ 70 per cent of animals were then radially or concentrically symmetrical; those with bilateral symmetry are often totally absent.

Some of the more bizarre forms exhibit radial symmetries that today are very rare or non-existent. Thus, an homogeneous group with primary 3-fold symmetry is recognized, and forms with 4- and 7-fold ordering are also common. A particularly important group, according to Fedonkin, is that in which the symmetry remains radial, but the order (number of arms, gastral cavities, and so forth) actually increases during growth. A possible insight into metazoan evolution comes from fossils such as the late Precambrian *Dickinsonia* which at first sight are bilaterally symmetrical but on closer examination have different numbers of segments on the left- and right-hand sides. Fedonkin terms this phenomenon "glide reflection" and believes that such forms would not be dynamically stable and so evolved to become properly bilateral. He envisages a major evolutionary pattern, recorded in the Vendian rocks, of a gradual change from the dominance of concentric and radial organization, through variable-order radial symmetry and glide reflection, to bilateral segmentation (Fig. 2). A. Seilacher (Universität Tübingen) and others dispute this view, finding no evidence that *Dickinsonia* had a mouth or a gut or any semblance of a metazoan digestive system. Consequently they believe such extraordinary fossils represent not the dawn of metazoans but perhaps a completely different kingdom. □

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Nitrogen fixation

Differentiating cyanobacteria rearrange their *nif* genes

from William D.P. Stewart

CYANOBACTERIA (blue-green algae) are oxygenic photosynthetic prokaryotes, many strains of which fix N_2 and which probably dominated the Earth's biota during the middle Precambrian, 2,500–570 million years ago. On page 419 of this issue, J.W. Golden, S.J. Robinson and R. Haselkorn report that, despite their ancient origin, the N_2-fixing photosynthetic cyanobacteria possess a molecular complexity that belies their morphological simplicity[1].

Although long regarded as algae, mainly because of their pigment composition and mode of oxygenic photosynthesis, cyanobacteria are probably more akin to prokaryotic bacteria than to eukaryotic chlorophyllous plants, and are seldom still classified as blue-green algae. They are most noticeable in extreme environments, for example in parts of Antarctica where they may form 'algal peat', in hot desert regions including parts of the Sahel, on bare rock surfaces, in hot-spring regions and in living stromatolites. Cyanobacteria are also important components of the marine phytoplankton and they, or more probably their colourless analogues, are components of the microflora of the hydrothermal vent regions of the Galapagos Rift. In many parts of the world, but particularly in south-east Asia where fertilizer nitrogen is not readily available, cyanobacteria are important providers of biologically fixed nitrogen for the growth of the rice plant[2,3].

It is partly because of their unique ability to fix N_2 while photosynthesizing in the manner of higher plants that the cyanobacteria have recently aroused the attention of molecular biologists. Their study may, in the long term, have relevance to the possibility of introducing the genes responsible for N_2-fixation (the *nif* genes) into the plastids, whether chlorophyllous or not, of higher plants. They have also gained attention as possibly useful models for developmental molecular biologists because many forms occur as simple unbranched filaments with a maximum of three cell types: heterocysts, the sites of N_2 fixation; akinetes, which are perennial; and vegetative cells, from which akinetes and heterocysts develop[4]. Haselkorn and co-workers now add to the scientific attractions of cyanobacteria by showing that they possess a so-far unique capacity to rearrange some of their N_2-fixing genes, notably two that encode the major components of nitrogenase.

The mechanism that allows the O_2-sensitive nitrogenase to function in oxygenic cyanobacteria such as *Anabaena* was for long unknown. It was then discovered[3,5,6] that the peculiar, empty-looking heterocysts[7], which occur in most N_2-fixing cyanobacteria, are the loci of nitrogenase activity in air and in the light, and that by various biochemical modifications, they provide an anaerobic microenvironment in which nitrogenase is synthesized and is functional.

A drawback, until recently[8], to the detailed genetic analysis of N_2-fixation and heterocyst production in cyanobacteria has been the fact that although mutants of cyanobacteria are readily obtainable, there has existed no good system for the transfer, in the laboratory, of genes into heterocystous cyanobacteria. Thus, genetic analysis by complementation of cyanobacterial mutants has not been possible. An alternative approach, used by Haselkorn and colleagues, is to use the *nif* genes of the enteric bacterium *Klebsiella* as probes for those of *Anabaena*. In *Klebsiella* there are 17 *nif* genes, organized into seven or eight transcriptional units and arranged in a cluster occupying about 23 kilobases of DNA and located near the genes for histidine biosynthesis (see ref. 9). The genes that encode the major nitrogenase components — the iron protein and the iron–molybdenum protein — are *nif K*, *nif D* and *nif H*. The iron protein is composed of two identical subunits, both encoded by *nif H*. The iron–molybdenum protein contains one pair of identical subunits encoded by *nif K* and another by *nif D*. Although the two proteins do not fix N_2 alone, in combination they may do so in the presence of a source of reductant, Mg^{2+} and ATP, and in the absence of O_2 (see ref. 10).

Haselkorn and his colleagues have already shown that, while the *nif K*, *D* and *H* genes are clustered in *Klebsiella* and some other N_2-fixing organisms, in DNA extracted from filaments of *Anabaena* 7120 the *nif D* and *nif H* genes are contigous but separated from *nif K* by about 11 kilobases. The Chicago group has

now compared the arrangement of the *nif K, D* and *H* genes in whole filaments of *Anabaena* with that in heterocysts during their differentiation from vegetative cells. While confirming that *nif K* is separated from *nif D/H* in vegetative cells, they show that a gene rearrangement occurs within the heterocyst at a late stage in differentiation. What happens is that the 11-kilobase intervening DNA sequence between *nif D/H* and *nif K* is excised, so that the three genes become clustered and function as a single transcriptional unit, with nitrogenase structural proteins being synthesized, and nitrogenase activity commencing, within the heterocysts. The excised portion remains in the heterocyst as a circular molecule of unknown function.

These findings are of interest not only because they are unique but because they emphasize the usefulness of cyanobacteria for the study of differentiation. The heterocysts of cyanobacteria such as *Anabaena*, show a distinct spatial pattern; between 8 and 10 per cent of the total cells along a filament are heterocysts and new heterocysts develop equidistantly from two existing heterocysts. That is, in some as yet unknown way, existing heterocysts may regulate the spacing pattern of new heterocysts (there is no evidence that nitrogenase is involved in the process). Furthermore, in organisms such as *Nostoc* 7524 there is evidence that with certain external (and no doubt internal) conditions, a cell that normally develops into a heterocyst develops instead into an akinete[12]. The molecular events leading to the cessation of cell division and determining whether a differentiating cell regresses, or further differentiates, and if so into which cell type, is an intriguing area of molecular biology that has relevance not only to nitrogen fixation but to our basic understanding of cellular developmental biology. At a time when the UK Agricultural and Food Research Council is examining N_2-fixation research, the new results with cyanobacteria will, perhaps, emphasize the merits of scientific diversity in this field. □

1 Golden, J W Robinson, S J & Haselkorn, R. *Nature* 314, 419 (1985)
2 Fogg, G E *et al The Blue-green Algae* (Academic, London, 1973)
3 Stewart, W D P *A Rev Microbiol* 34, 497 (1980)
4 Carr, N G & Whitton, B A (eds) *The Biology of Cyanobacteria* 2nd edn (Blackwells, Oxford, 1982)
5 Stewart, W D P, Haystead, A & Pearson, H W *Nature* 224, 226 (1969)
6 Bothe, H *et al* in *Advances in Nitrogen Fixation Research* (eds Veeger, C & Newton, W E) 199 (Nijhoff/Junk, The Hague, 1984)
7 Fogg, G E *Ann Bot* 13, 241 (1949)
8 Wolk, C P *et al Proc natn Acad Sci U S A* 81, 1561 (1984)
9 Dixon, R A *J gen Microbiol* 130, 2745 (1984)
10 Ludden, P W & Burris, J E (eds) *Nitrogen Fixation and CO_2 Metabolism* (Elsevier, New York, 1985)
11 Haselkorn, R *et al Ann Microbiol (Inst Pasteur)* 134B, 181 (1983)
12 Sutherland, J M, Herdman, M & Stewart, W D P *J gen Microbiol* 115, 273 (1979)

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Scale-invariant phenomena

Viscous fingers and fractal growth

from L.M. Sander

A RECENT article in Nature by Nittmann, Daccord, and Stanley[1] is a particularly instructive example of the power of basic science to find connections between apparently unrelated natural phenomena. It is an experimental account of the shape of the interface between two fluids with relevance to oil-field technology. The shape is shown to belong to a class that includes the shapes of electrolyte deposits[2,3], metals and dielectric breakdown structures such as lightning[4]. The common feature of the objects is that they are all scale-invariant fractals of the same fractal dimension[5].

The experiment of Nittmann *et al.* deals with the long-known fact that when a relatively non-viscous fluid is forced against a viscous fluid an instability results: 'fingers' of the less viscous fluid intrude into the other. For example, when carbon dioxide is pumped into an oil field, the efficiency of oil recovery is not very large because instead of the oil being pushed uniformly across the field, it is broken up by the branching pattern of the fingers. The usual laboratory realization of the system is a Hele-Shaw cell: water is pumped into a long channel in which oil is trapped between closely-spaced parallel plates. In the essentially two-dimensional region between the plates, fingering is easily seen.

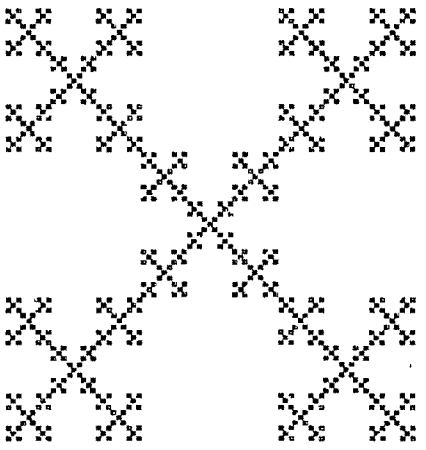
In past experiments, the shape of the fingers has been dominated by the interfacial tension between the two fluids, which tends to favour coarse fingers. Typically, one finger is formed with a width near to that of the cell. But, in an oil field, the region of interest is much bigger than a single finger. To simulate the oil field in their experiments, the essential step of Nittmann *et al.* was to use two fluids whose interfacial tension is essentially zero — water and an aqueous polymer solution that has a slow rate of mixing with water. In addition, the polymer solution has very high effective viscosity. Both these factors favour the formation of small fingers.

It is with respect to the sprawling, many-branched structures formed as the small fingers of water advance into the polymer solution that connections can be made to disparate phenomena. For, as Paterson has pointed out[7], the fundamental instability in this situation is the same as in other systems where diffusion limits growth. Here, fingers grow because it is easier for viscous fluid to flow away from a sharp tip than a flat interface. In dielectric breakdown, the electric field is largest at the end of the breakdown channel, leading to further extension at the point. In electrolyte deposition, new ions more easily find sharp tips than flat areas; the tips thus extend.

For instability dominated growth, the unifying framework is the diffusion-limited aggregation (DLA) model[8]. In this idealization of growth by diffusion, which is used in computer simulations, a particle walks randomly until it finds a nucleation centre, where it sticks. Then another particle is released and walks until it finds either the centre or the other particle and sticks. And so on. The remarkable result of the simulations is that the structures they form are scale-invariant and fractal.

Scale-invariant objects look the 'same' at any magnification. The disorderly structures already mentioned share this property with other natural objects, such as coastlines. The figure gives a simplified example, generated by an obvious rule, which looks the same at a discrete set of magnifications. In order to see what is meant by a fractal, we define D, the fractal dimensionality[5]. Cover the object in the figure with a set of disks of radius r. This takes a certain number of disks, $N(r)$. Now change r and see how N changes. Almost always we find $N(r) = Cr^{-D}$ where C is a constant. For a straight line, $D=1$ and for a square, $D=2$; in these cases D corresponds to our usual ideas of dimensionality. But for the illustrated figure, dividing the radius by 3 multiplies N by 5. Thus $D=\log(5)/\log(3)$. An object whose D is not given by its topology is a fractal.

Electrolyte deposits, dielectric breakdown stuctures and DLA clusters all have a measured D of about 1.7 in two dimensions of space. Their fractal properties can be studied using the simple DLA model. The fingers of Nittmann *et al.* have a smaller D, but the authors show that this is probably the effect of the finite width of their channel. All of these structures belong to the same universality class which seems to be that associated with growth far from equilibrium. Another class, corresponding to DLA in three dimensions (with $D=2.4$), has been seen in electolyte deposition[2]. Still another class appears in



the description of flow through porous materials — 'the percolation problem'.

The experiment of Nittmann *et al.* is also significant in that it gives insight into the minimum requirements of a DLA-like structure. Although DLA seems quite simple, it has defied complete analysis. A major point of contention is whether noise due to the discrete nature of the particles making up the cluster dominates the dynamics. Dielectric breakdown structures have links of more-or-less fixed length and electrolytic deposits are polycrystalline. Experiments on these systems shed no light on the question. But the fluid system of Nittman *et al.* is continuous. Apparently, discreteness is not essential to make a DLA-like fractal. There has been speculation that this would indicate that DLA is an example of chaos. In practical terms it may mean that solidification far from equilibrium, which can also be mapped onto DLA, could give rise to fractals — for example, if a snowflake were allowed to grow very large, it would probably be fractal, too. □

1 Nittmann, J , Daccord, G & Stanley, H E *Nature* 314, 141 (1985)
2 Brady, R M & Ball, R C *Nature* 309, 225 (1984)
3 Matsushita M , Sano, M , Hayakawa, Y , Honjo, H & Sawada, Y *Phys Rev Lett* 53, 286 (1984)
4 Niemeyer, L , Pietronero, L & Wiesmann, H J *Phys Rev Lett* 52, 1033 (1984)
5 Mandelbrot, B *The Fractal Geometry of Nature* (Freeman, San Francisco, 1982)
6. Saffman, P G & Taylor, G I *Proc R Soc* A245, 312 (1958)
7 Paterson, L *Phys Rev Lett* 52, 1621 (1984)
8 Witten, T A & Sander, L M *Phys Rev Lett* 47, 1499 (1981), *Phys Rev* B27, 5685 (1983)

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100 Years Ago

THE following account, we learn from *Science*, of unusual phenomena was received, March 10, at the Hydrographic Office, Washington, from the branch office in San Francisco. The barque *Innerwich*, Capt. Waters, has just arrived at Victoria from Yokohama. At midnight of February 24, in latitude 37° north, longitude 170° 15′ east, the captain was aroused by the mate, and went on deck to find the sky changing to a fiery red. All at once a large mass of fire appeared over the vessel, completely blinding the spectators; and, as it fell into the sea some fifty yards to leeward, it caused a hissing sound, which was heard above the blast, and made the vessel quiver from stem to stern Hardly had this disappeared when a lowering mass of white foam was seen rapidly approaching the vessel. The barque was struck flat aback; but, before there was time to touch a brace, the sails had filled again, and the roaring white sea had passed ahead The master declares that the awfulness of the sight was beyond description and considers that the ship had a narrow escape from destruction.

From *Nature* **31** 514, 2 April 1885.

Oceanography

Sonar classification of sea beds

from M.L Somers

ALTHOUGH side-scan solar is used widely in both scientific and engineering investigations of the sea bed, the task of extracting a numerical classification of sea beds from the backscattered sound has proved intractable. But an experienced human observer who has had access to the results of comparisons between sonographs and physical samples can rapidly make accurate qualitative classifications, so there must be some quality of the backscattering that gives the necessary clues. On page 426 of this issue, Z. Reut, N.G. Pace and M.J.P. Heaton offer a solution to the problem and a method of classification into six sea-bed types — mud, sand, clay, gravel, stones and boulders.

The backscattering of sound from the sea bed is a complicated function of the grazing angle of the sonar signal and the roughness of the sea bed, with further complications thrown in by the acoustic impedance of the sea-bed material: if the ratio of sea bed to water impedance is close to unity (as in the case of mud), then there is appreciable penetration of sound into the sea bed and the possible production of a boundary wave, which can interact with the incident wave, profoundly modifying the backscattering process (see Somers, M.L. and Stubbs, A.R. *IEE Proc.* **131**, Pt F; 1984). To circumvent these problems, Reut *et al.* have concentrated on the rate of fluctuation of the backscattered sound over short periods of time. Their method is based on the observation that the fluctuations generally become more rapid as the ground gets rougher. There are, of course, large differences in total energy, and the peak-to-valley ratios can actually be greater over a soft or smooth sea bed than over a rough one, but the rate of fluctuation is still generally less than if the sea bed is rough.

The method requires fairly careful normalization, for which Reut *et al.* have chosen to use cepstrum analysis, an outline of which is given in their paper. By taking the mean of the power spectrum, all considerations of phase can be ignored, so ensuring that when the inverse transform is taken the result peaks at, or near to, zero time delay. The effect of taking logs before doing the inverse transform is to de-emphasize any d.c. level in the signal, which shows up as a large peak at zero frequency in the power spectrum. The two-power cepstrum integral parameters give a measure of how closely the cepstrum is confined to low time-lag values.

Justification of the method comes both from the foregoing qualitative arguments that can be mustered in its favour and from the fact that it seems to work — 120 sea-bed areas are correctly classified into the six types with the only overlap being between gravel and stone types. A similar result could probably be obtained by applying automatic gain control, removing the mean and taking the auto-correlation function, though this would possibly be computationally less attractive.

It would be interesting to see the method applied to a wider selection of sea-bed echoes, using a number of frequencies instead of the single 48-kHz frequency used by the authors. An application which comes to mind concerns the problem of mapping and assessing fields of manganese nodules in oceans that are several thousand metres deep. This will require low-frequency sound, such as used in the long-range side-scan sonar, GLORIA II. Manganese nodule fields occur very widely in the Abyssal Plain regions of the deep ocean and should be visible on a GLORIA record. If so, it might at least be possible to delineate the boundaries of the fields, and perhaps even to give a measure of nodule abundance.

Side-scan sonar requires oblique incidence for its best visual effect, so that depth/range ratios need to be less than about 0.2. But most systems working from the surface in deep water, or at high frequencies in shallow water, operate at near-vertical incidence and the backscattering process is somewhat different. It will be interesting to see how the classification system of Reut *et al.* performs in such circumstances.

Turning from their concern with problems of propagation and surface reverberation, the underwater-acoustics community is devoting increasing attention to sonar scattering. Many theoretical papers and some reporting measurements or equipment will be given at the International Conference on Scattering Phenomena on 2-3 April at the Admiralty Research Establishment, Portland, UK. Various applications of the analysis of sea-bed backscattering, both civil and military, are being explored. Of the civil applications, many involve the estimation of resources. Manganese nodules have already been mentioned. Gravel provides a second example. Over ten per cent of the gravel used in the United Kingdom is dredged from the sea bed, and a rapid and reliable means of detecting suitable gravel fields would be of considerable value. The accuracy of acoustic logs for ships that measure their speed by observing the Doppler shift of sound backscattered from the sea bed should also be improved by application of the new system of classification. □

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Ramsey's silicate core revisited

SIR — The case for believing that the Earth has an iron core was called the "classical view" by Birch [1] when, in 1952 he reviewed and rejected Ramsey's earlier argument [2-4] that "the mantle and core are not chemically distinct". Briefly, Ramsey's proposal was that, because the terrestrial planets were not originally of the same composition (as reflected by lack of correspondence in relative mass and mean density), then the amount of iron in the cores of the planets must be out of correspondence, so that the iron core hypothesis must be rejected. Instead, Ramsey suggested that the core should be regarded as a metallic phase of a silicate such as olivine.

The arguments which led Birch to reject Ramsey's theory are still valid. One was that elements with a low atomic number would not reach the required density even at core pressures. On the basis of theory, he concluded "...that a compound such as Mg_2SiO_4, of mean atomic number equal to 10, would reach a density of 10 only at a pressure ten times as great as the pressure of the core boundary, even in the fully ionized conditions".

In 1952, there were no laboratory data on iron at the conditions of the Earth's core, but, by 1960, because of advances in shock-wave physics, experimental data [5,6] were sufficient for the subject to be examined again. In 1963, Birch [7] reviewed the experimental evidence on shocked iron, and concluded that the sound velocity of silicates at core pressure could not possibly be as low as the sound velocities of the core reported by seismologists. Thus, experimental evidence backed up his earlier conclusions.

The shock-wave evidence accumulated since 1963 has repeatedly verified Birch's position, but this laboratory evidence is ignored when the proposal of a silicate core is revived, as it has been from time to time, most recently in *Nature* [8,9].

A *News and Views* article stated that R. Lyttleton is "entirely right to carry a torch for Ramsey's theory, which is in a category too neat not to be true". The view was expressed that the chief objection to Ramsey's hypothesis was that the experiments of high pressure geophysics had failed to verify Ramsey's hypothesis, but might be "irrelevant", although why these important experiments are irrelevant was not explained. It, therefore, is timely to review the most important experimental evidence in favour of the iron core and against the silicate core.

The chief objection to Ramsey's hypothesis is not that the shock-wave experiments have failed to find the postulated phase transition in olivine and similar minerals, although that is significant, but that shock-wave experiments have shown that pure iron at inner-core pressure and temperature has the mechanical properties that seismologists find for the inner core.

It is important to distinguish the properties of the inner core from those of the outer core; not only is one solid and the other liquid, but the former is virtually pure iron and the latter, iron diluted by lighter elements.

The argument assembled by Birch in 1963 has been largely overworked in the past decade. He found a correspondence between the velocity of sound found by seismologists and by shock-wave determinations.

The seismically determined shear and longitudinal sound velocity, V_P and V_S respectively, determined in the Earth at every depth, is related to the bulk modulus and density, K and ϱ, by $\phi = (K/\varrho)^{1/2} = (V_P^2 - 4/3\ V_S^2)^{1/2}$. Now ϕ is the bulk sound velocity, also given by $(\delta P/\delta\varrho)^{1/2}$, where P is pressure measured by shock waves. By measuring $(\delta P/\delta\varrho)^{1/2}$ on iron at core pressures and temperatures, using shock waves, comparisons are made between the value of ϱ for iron and the seismic ϕ in the core. Birch pointed out that the velocity of sound has an important relationship to density, forming a pattern that has come to be known as velocity-density systematics, and which has been widely used to estimate unknown compositions in the Earth's interior from seismological data. For metals under shock waves, the pattern relating the bulk sound velocity and density is dependent on the atomic number. The larger the atomic number, the smaller the velocity for a given density. Birch's (1963) graph, modified by additions, is reproduced as Fig.1.

In the period, 1965-75, velocity-density studies showed that a phase transition in an oxide such as olivine increases both K and ϱ, but that K increases as a power of ϱ ($K \sim \varrho^x$, $x = 4.5$) [10-13]. Thus, the ϕ and ϱ curve for the high pressure phase in a silicate is merely a projection of the ϕ-ϱ trajectory for the low pressure phase, as shown (Fig.1) by the dunite and olivine curves [14]. Because all phases of dunite lie on a line in the field of low atomic numbers, it was generally concluded that if Ramsey's theory were correct, the value of $\phi = \sqrt{K/\varrho}$ of a silicate would have to be quite large at the densities found in the core, exceeding perhaps 20 km s^{-1}. In brief, the bulk sound velocity for the core is far too low to be compatible with a silicate. Whatever the core is composed of, it must have an average atomic number near 25 or 26.

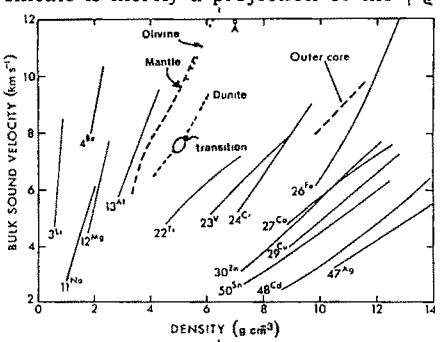


Fig.1 Bulk sound velocity $\phi = (\delta P/\delta\varrho)^{1/2}$ plotted against density ϱ along the Hugoniot compression curves for metals of low atomic number [5]. The heavy dashed lines show the bulk sound velocity, $\phi = (V_P^2 - 4/3\ V_S^2)^{1/2}$, of the mantle and core as determined from seismology. Compression curves are given for dunite [14] through a transition at $\varrho = 4.5$ g cm^{-2}; and for dunite (A) at a very high shock pressure [6]. The ϕ versus ϱ curve for shocked olivine is also shown. Silicates cluster near the mantle curve, near an effective atomic number of 14. The outer core curve is in the vicinity of metals with an effective number near 25 (modified from ref 7).

Moreover, the density changes in dunite are small compared with the differences between mantle to core, as is the case for all silicates so far measured by shock-wave experimentation.

Refinements of shock-wave data on iron show that the density of the outer core is not quite as large as expected for pure iron [15], so the outer core is presumed to be iron with a small concentration of solutes (as inferred by Birch [7] from his equation of state).

Birch's 1952 and 1963 papers convinced most Earth scientists that the Earth's core is iron diluted with a small concentration of solutes. The live issue in the field became that of what those solutes could be.

Experiment after experiment has verified Birch's concept (Fig.1). Refinements of shock-wave measurements have shown that the candidate solutes in the liquid core are S, O, or Si (refs 16-19). For example, Ahrens [16] used his shock-wave experiments to demonstrate the relation of the density of Fe-S compounds to the density of the outer core as determined from seismic data. By the early 1980s, shock-wave experiments of improved resolution had shown that the data for pure iron at core conditions corresponds to the seismically determined density of the inner core [16,19]. The Earth's inner temperatures had become better constrained by this time, so that the mechanical properties of iron (ϕ, K and ϱ) could be computed; Jeanloz [19] found that they agreed with the known properties of the inner core.

By 1982, the hypothesis that the inner core has the same physical properties as pure iron had been widely accepted; the question that remained was that of from which phase of pure iron it is constituted. There were two possibilities, ε-iron (hexagonal close-packed) or γ-iron (face-centred cubic). Several theoreticians favoured γ-iron and computed physical properties based upon this choice [20-22]. But in 1980, Brown and McQueen [23,24], using an elegant modification of shock-wave techniques, were able to measure the longitudinal velocity, V_P, of iron at core pressures. The significance of this measurement is that, when the shock wave encounters the liquid state, V_P abruptly decreases to ϕ, since the shear velocity, V_S, vanishes, with the result that the pressure and temperature of a point on the fusion curve of iron are determined. They found [25] melting

at 243 ± 2 GPa and the ε-γ transition at the lower pressure of 200 ± 2 GPa. The corresponding temperatures are 5,000-6,000 K and 4,400 ± 300 K.

A simple extrapolation (from 243 to 350 GPa) of the measured V_p to inner core pressures, after correcting for the temperature expected, indicates that the V_p of pure iron at core conditions matches the seismic V_p of the inner core (Fig.2). Since Poisson's ratio is determined by ϕ and V_p, a good match is obtained between pure iron at inner core density and that reported by seismologists for the inner core. Thus, we have agreement for the values of ϕ, σ, V_p, and ϱ when comparing shock-wave measurements for pure iron with those inferred by seismologists for the Earth's inner core.

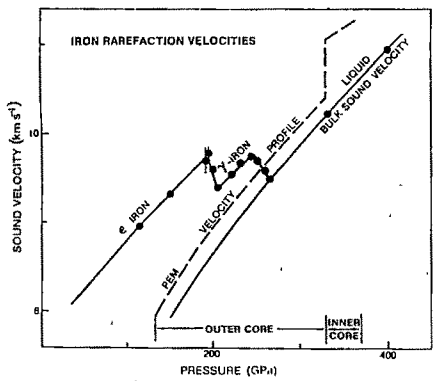


Fig.2 The plot of compressional velocity V_P against pressure is shown by solid circles for shocked iron along the Hugoniot. At lower pressure, shocked iron is in the ε-phase; at 243 GPa, it transforms by a solid-solid transition to the γ-phase; and at 250 GPa, it further transforms to the liquid phase. The liquid phase is indicated by the fact that V_P has the same value as the bulk sound velocity. The dashed line shows the plot of V_P against pressure for the outer core and the inner core (called PEM), as determined from seismology[35]. Note that V_P for shocked ε-iron extrapolates to higher pressure values of V_P for the inner core (modified from Fig.1 of ref.24).

The convergence of current research on the view that the inner core is ε-phase iron is supported by the extrapolation of the physical properties of the ε and γ phases to inner-core pressure; the ε-phase gives better agreement than the γ-phase[25]. The choice of ε-iron is not universally accepted however; for example, Spiliopoulos and Stacey[22] still hold out for γ-iron as the sole constituent of the inner core, using theoretical arguments on the behaviour of phase boundaries at high pressure.

Cosmochemists require alloys of iron for the cores of planets[26-29] and many theories suggest a steady cooling of their interiors. Thus, there is fractional crystallization by a moving solid-liquid interface as the inner solid core slowly grows, tending to concentrate the solute in the outer liquid part of the core[30]. This releases potential energy and is widely believed to be the energy source of the core dynamo that generates the Earth's magnetic field.

The shock-wave data of iron are thus consistent with a clear model of the core: The inner core is growing, the impurities are concentrated in the outer core, the Earth is cooling and the dynamo is gravitationally driven.

The solutes in the outer core are atoms lighter than iron, according to cosmochemistry[26,28], so the density of the outer core is less than that of pure iron. Thermodynamically, it does not matter much what these light atoms are, for so long as the concentration is small, different solutes will equally well depress the density of pure iron to give the measured seismic velocity and density of the outer core. The detailed composition of the outer core is, of course, of intense interest to cosmochemists and cosmologists, but whether the solute is sulfur[18], oxygen[28], silicon or hydrogen[31], or combinations thereof, does not affect the conclusion that the Earth's outer core is predominantly iron, and that the inner core is essentially pure iron.

The details of the phase diagram of iron, up to inner-core pressures and temperatures, have been evolving for twelve years[32-34]. I accept the conclusion of Brown and McQueen that ε-iron constitutes the inner core[24,25], which means that the ε-γ-liquid triple point is at a pressure below that of the boundary between the inner and outer core. The phase diagram of iron (Fig.3) is slightly adapted from ref.34. A complete description of the phase diagram of iron at inner core conditions is presented elsewhere[35]. There the thermodynamically derived density of iron at conditions at the top of the inner core is found to be 13.0 g cm^{-3}, compared with the seismically determined density of 12.86 g cm^{-3} by one report[36], and 13.4 by another report[37].

Thus, in the past twenty years, evidence for the iron core hypothesis has mounted to the point that it is overwhelming. Most now involved in research on the properties of the Earth's core find Ramsey's 1948 hypothesis[2], and Lyttleton's revival of it, somewhat quaint. To restore the acceptance of Ramsey's idea of a silicate core would require not only the verified proof of the existence of a high-pressure phase change of a silicate wherein the density is doubled, but also proof that all the physical properties (K, ϱ, σ, V_p) of such a high pressure phase duplicate the properties of the inner core.

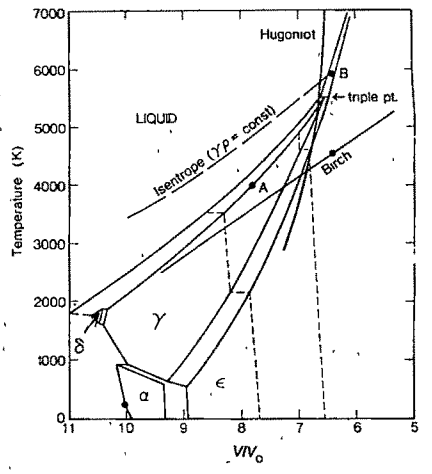


Fig.3 The volume-temperature phase diagram of iron (adapted from Fig.5 of ref.34). The phase boundaries are concave upward, and connect three triple points. B marks the inner-outer core boundary pressure, and A marks the core-mantle boundary pressure. The two broken lines are isobars at 100 and 250 GPa. The upper triple point (γ-ε-liquid) is placed at slightly above 250 GPa. Birch's 1972 extrapolation[32], based on an assumed linear T_m versus V/V relationship, is shown. The temperatures along the solidus are determined by the data represented by Fig.2.

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1. Birch, F *J. geophys Res* **57**, 227-285 (1952)
2 Ramsey, W H *Mon Not R. Astr Soc.* **108**, 403-413 (1948)
3 Ramsey, W H. *Mon Not Roy Asron Soc., Geophys Suppl* **5**, 409-426 (1949)
4 Ramsey, W H *Mon Not. R Astr Soc , Geophys Suppl* **6**, 42-49 (1950)
5 McQueen, R G & Marsh, S P *J appl Phys.* **31**, 1253-1263 (1960)
6 Altschuler, L.V & Kormer, S.B. *Bull acad sci ; USSR, Geophys Ser* **1**, 18-23 (1961).
7. Birch, F , in *Solids Under Pressure* (eds Paul, W & Warschauer, D) 137-162 (McGraw Hill, New York, 1963)
8 *Nature, News and Views* **300**, 681 (1982)
9 Lyttleton, R A *Proc R. Soc* **A287**, 471-493 (1965).
10 Anderson, O L *J. geophys Res* **71**, 4963-4971 (1966)
11. Anderson, D L *Geophys. J R Astr Soc* **13**, 9-16 (1967)
12 Shankland, T J *J. geophys Res* **77**, 3750-3760 (1972)
13 Davies, G F *Earth Planet Sci Lett* **22**, 239-41 (1974)
14 McQueen, R G , Marsh, S P & Fritz, J N *J. geophys Res.* **72**, 4999-5036 (1967)
15 Ahrens, T J *J geophys Res* **84**, 985-998 (1979)
16 Ahrens, T J *Science* **207**, 1035-1040 (1980)
17 McQueen, R G & Marsh, S P *J geophys Res.* **71**, 1751-1956 (1966)
18. Stevenson, D J *Science* **214**, 611-619 (1981)
19. Jeanloz, R *J geophys. Res* **84**, 6059-6069 (1979)
20 Bukowinski, M *Phys Earth planet. Inter* **14**, 333-343 (1977)
21 Stacey, F.D *Phys Earth Planet Inter* **15**, 341-348 (1977)
22 Spiliopoulos, S & Stacey, F D *J Geodynamics* **1**, 61-77 (1984).
23 Brown, M J & McQueen, R *Geophys Res Lett* **7**, 533-536 (1980)
24 Brown, M J & McQueen, R in *High Pressure Research in Geophysics* (eds Akimoto, A & Manghnani, M) 611-625 (Center of Academic Publications, Tokyo, 1982), *Adv Earth planet Sci* **12**, (1982)
25. Brown, M.J. & McQueen, R *J. geophys Res* (in the press)
26 Brett, R *Rev Geophys Space Phys.* **14**, 375-383 (1976)
27 Murthy, V R & Hall, H T *Phys Earth planet Inter* **6**, 123-130 (1972)
28 Ringwood, A E *Geochem J* **11**, 111-135 (1977)
29 Ito, K *Geochem J* **10**, 59-64 (1976)
30. Verhoogen, J *Geophys J R Astr Soc* **27**, 1-14 (1961); *Energetics of the Earth* (National Academy of Sciences).
31 Fukei, Y , Fukizawa, A , Watanabe, K & Ameno, M *Jap J appl Phys* **21**, L318-L320 (1982)
32. Birch, F *Geophys J. R. Astr Soc* **29**, 373-387 (1972)
33 Liu, L *Geophys J. R Astr Soc* **43**, 697-705 (1979)
34. Anderson, O L *Phil Trans. R Soc* **A 306**, 21-35 (1982).
35 Anderson, O.L *Q J R. Astr Soc* , (in the press)
36 Dziewonski, A M & Anderson, O L *Phys Earth planet. Inter* **25**, 297-356 (1981)
37 Bolt, B , *Inside the Earth*, Table 4 (Freeman, San Francisco, 1982)

Unification of forces and particles in superstring theories

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Superstring field theories have emerged as potentially consistent quantum field theories that unify gravity with the other fundamental forces in an almost unique manner. They are based on the dynamics of string-like fundamental quanta rather than the point-like quanta of more familiar relativistic 'point field theories' such as Yang–Mills gauge theory or general relativity. In these theories the observed fundamental particles, such as the leptons and quarks, may arise as the ground states of a string. This amounts to a profound generalization of the conventional field theory framework.

IN this article I will review the structure of superstring theories developed over the past five years[1-4]. I will describe in particular how these theories are restricted by requiring that they be consistent with quantum mechanics. Recently we have understood how these restrictions (which cannot be satisfied by point field theories) constrain the possible internal symmetry groups of superstring theories to just two possibilities, namely the special orthogonal group SO(32) or the product of two exceptional groups $E_8 \times E_8$ (ref. 5). The fact that the internal symmetry is determined almost uniquely by requiring the quantum consistency of a theory containing gravity is a novel development. The hope is that this will provide a realistic unified theory that will explain experimental observations with few or no free parameters. I will outline some interesting developments in this direction.

Grand unification and supersymmetry

In the past decade a variety of Grand Unified Theories (GUTs) have been developed to unify the strong and the electro-weak interactions at an energy scale of $\sim 10^{16}$ Gev. GUTs are gauge invariant (Yang-Mills) point field theories which do not attempt to incorporate the gravitational force, and so there are few theoretical constraints on the possible symmetry groups. These groups as well as a large number of undetermined adjustable parameters are therefore chosen to account for observations as well as possible. The most favoured GUTs are based on the special unitary group SU(5), the special orthogonal group SO(10), or the exceptional group E_6. In such schemes the quarks and leptons are unified into families. The quarks and leptons so far discovered make up three of these families but the question of how many families there are in total is a matter of speculation in the absence of a fundamental theory.

The observed elementary particles have masses that are tiny compared with the natural mass scale in a GUT of $\sim 10^{16}$ GeV. To a first approximation these masses are taken to be zero and the observed non-zero mass values arise from small perturbative corrections. Supersymmetry is an important ingredient in GUTs that ensures the possibility of predicting reasonable masses for these particles. It is a symmetry that relates bosons to fermions rather as internal symmetries relate groups of particles. However, as supersymmetry relates particles of different spins it is not an internal symmetry but an enlargement of the Poincaré group (the group of Lorentz transformations and translations) to the 'super-Poincaré group'. This amounts to an extension of space-time to 'superspace' that includes extra spinorial anticommuting coordinates as well as ordinary coordinates. Although theoretically appealing, the existence of sypersymmetry in nature has yet to be established experimentally. It probably requires there to be a host of particles (known as squarks, sleptons, Winos, Zinos, ...) which have not yet been discovered

Supergravity theories are point field theories that incorporate local (that is, gauged) supersymmetry, thereby enlarging Einstein's theory of general relativity. In recent years such theories have been the focus of much research in constructing quantum theories containing gravity as well as unifying gravity with the other forces. There are fundamental problems in uniting quantum mechanics and general relativity that are associated with effects at the Planck scale (10^{-35} m). Despite early optimism that incorporating supersymmetry would resolve these problems and also unify the interactions in a geometrically appealing manner, it now seems probable that none of the supergravity theories will lead to a consistent quantum theory.

However, there is now the possibility of predicting an almost unique unified theory as a low-energy approximation to a consistent superstring theory (where the term 'low energy' means an energy low compared with the Planck scale, 10^{19} GeV).

What are superstrings?

The behaviour of a relativistic string moving in space-time differs significantly from that of a structureless point particle. Unlike a point particle, a classical relativistic string has an infinite number of vibrational modes with arbitrarily high frequencies and angular momenta. This means that in the quantum theory a single string has an infinite number of states with masses and spins which increase without limit. The scale for the mass splitting between these states is set by the string tension T (with dimension (mass)^2 in natural units). String theories were developed originally in the early 1970s as models for strong interaction physics (see refs 6, 7 for reviews). For example, a meson was thought of as a string with a quark attached to one end and an antiquark to the other. T was then supposed to be ~ 1 GeV^2 and the excited states were interpreted as hadron resonances. The main theories of this type were the 'bosonic' string theory, which only described bosons, and the 'spinning' string theory, which incorporated fermions as well as bosons. These early string theories, however, had severe theoretical inconsistencies because the string ground state always turned out to have negative mass-squared (that is, it was a tachyon).

Superstring theories, which evolved from the spinning string theory, incorporate supersymmetry and do not suffer from the inconsistency of having tachyonic ground states. In fact, the ground states have zero mass and are simply the fundamental states of interesting supergravity point field theories. Superstring theories are therefore interpreted as theories that include gravity and the natural mass scale set by T is the Planck scale (that is, $T^{1/2} = 10^{19}$ GeV). The excited states are so massive that for many

purposes they can be taken to be infinitely heavy and the theory can be approximated by an effective point field theory of the massless states only. In other words, at energy scales well below the Planck scale (or distances $\gg 10^{-35}$ m) the string looks like a point. The quantum mechanical consistency of certain superstring field theories, however, depends crucially on the fact that at energies approaching the Planck scale the higher mass states are excited. The short distance structure therefore differs radically from that of any point field theory, such as Einstein's theory of gravity.

The classical dynamics of a relativistic string is described by an obvious generalization of the dynamics of a point particle. Just as a relativistic point particle moves along a world-line in space-time a string sweeps out a world-sheet as it moves. The obvious invariant action for a single point-particle is the length of its world-line and, similarly, the action for the simplest type of string is the area of its world-sheet[8-10]. For a superstring, the action is a generalization of the surface area to something like the area in superspace (which again requires additional spinorial dimensions to be adjoined to the space-time dimensions)[11].

The passage from classical mechanics to quantum mechanics turns out to impose severe constraints on any string theory. For example, all string theories contain massless spin-1 and spin-2 states which are associated with a Yang–Mills gauge boson and a graviton, respectively. Furthermore, the consistency of the quantum theory selects a special dimension for space-time (the 'critical' dimension). For the original (bosonic) string theory this was 26 dimensions, whereas superstring theories require 10-dimensional space-time. Just as in any theory containing gravity, the structure of space-time should be determined dynamically and so, to be of relevance to physics, the extra six dimensions must curl up and be very small (they must 'compactify'). This is analogous to the idea originally proposed by Kaluza and Klein[12,13] in the early days of general relativity as a way of unifying gravity with electromagnetism which has received renewed interest recently in the study of supergravity point field theories. However, in the context of any point field theory, the extra dimensions make the problems of the quantum infinities of the theory even worse than they already are in four dimensions. One of the remarkable aspects of the quantum superstring field theories is that certain of them seem to have no infinities at all, even though they are defined in 10 dimensions.

Restrictions on chiral theories

A very important physical constraint on any theory is that it must give rise to the observed 'chirality' of our (approximately) four-dimensional world, that is, the lack of invariance of the laws of physics under parity inversion. In the past few years it has become clear that obtaining a chiral four-dimensional world from a higher dimensional theory imposes severe restrictions on such theories[14,15]. It seems to be essential that the theory have chiral fermions in the higher dimensions (that is, in 10 dimensions for superstring theories) as well as a Yang–Mills gauge symmetry. In that case, the chirality of the higher-dimensional fermions can be maintained when the extra dimensions compactify, if the gauge fields twist up in a topologically non-trivial manner in the internal compact space. An example of such a non-trivial field configuration would be that of a magnetic monopole. A theory formulated in an odd number of dimensions cannot be chiral and so would seem to have little prospect of predicting sensible four-dimensional physical laws.

A theory with chiral particles will have terrible problems, however. The conservation of the Yang–Mills charges as well as the energy–momentum that is built into the classical theory may be violated in the quantum theory. Theories in which there is a quantum mechanical breakdown of such sacrosanct conservation laws are said to have 'anomalies'. These anomalies allow unphysical degrees of freedom (such as the longitudinal mode of the photon) to couple to the physical ones rendering the theory inconsistent. In 10 dimensions (and more generally in $4n+2$ dimensions), parity violation is an intrinsic property of a theory with chiral fermions (or self-dual bosons) independent of the Yang–Mills group G or to which representation of G the fermion belongs. (In contrast, in four dimensions a theory only violates parity if it contains chiral fermions in a complex representation of the gauge group.) For this reason, gravitational and Yang–Mills anomalies can generally be expected in any chiral 10-dimensional gauge theory. Requiring the absence of all anomalies leads to such stringent conditions that until recently it was thought that no interesting chiral theory could satisfy them. The results I shall describe show that for just two choices for the gauge group G it is possible to construct an anomaly-free theory. This requires a very intimate relationship between Yang–Mills and gravitational interactions, which looks somewhat contrived when viewed in terms of the effective point field theory that approximates the string at large distances However, this unification of the Yang–Mills and gravitational forces is one of the most striking aspects of string theories.

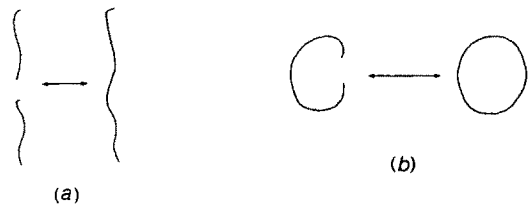


Fig. 1 The Yang–Mills interactions of strength g. *a*, Coupling between two open strings and a third open string (This interaction contains the usual interaction between three massless gauge bosons) *b*, The transition between an open and a closed string.

Classification of superstring theories

Type I theories describe the dynamics of open strings that have free endpoints. These strings carry quantum numbers in the n-dimensional defining representation of a classical group G = SO(n) or the symplectic group USp(n) at their endpoints (SU(n) leads to inconsistencies in the string field theory). This is similar to the way in which the quark quantum numbers were incorporated in the original string picture of mesons. It has not been understood how to incorporate the exceptional groups in this type of string theory. Although the string is locally invariant under two supersymmetries (that is, $N=2$), the free-end boundary conditions break this down to just one (that is, $N=1$) supersymmetry. The massless open-string states are the usual states of supersymmetric Yang–Mills theory in 10 dimensions with the gauge group G. Two open strings can interact when two ends touch and join to form one open string or, conversely, one string splits into two (Fig. 1*a*). An important corollary to this is that the two endpoints of a single string can join to form a single closed string so that type I theories necessarily contain a closed string sector (Fig. 1*b*). The massless states of a closed string form a supergravity multiplet (and do not carry the Yang–Mills quantum numbers).

This means that the effective field theory that emerges as the low energy approximation to type I theories is $N=1$ Yang–Mills coupled to $N=1$ supergravity. The fact that the existence of the Yang–Mills sector requires the existence of the gravitational sector is quite unfamiliar in ordinary point field theories. This unification of the Yang–Mills and gravitational forces leads to the relationship $\kappa = \text{const.} g^2 T$ between the gauge coupling constant, g, and the gravitational coupling (Newton's constant), κ.

Because these are chiral theories with a Yang–Mills gauge group, they may be of interest in describing observed physics for the reasons mentioned earlier. I will describe later how requiring the absence of all anomalies in type I theories restricts the choice of gauge groups to only one possibility—SO(32). I will also argue that, from the point of view of the low-energy point field theory, the group $E_8 \times E_8$ is also anomaly-free. This suggests that there might also be a way of incorporating it in a type I theory.

Type II theories only involve closed strings. These can have an 'orientation' associated with the fact that waves can run around the string in two possible directions. The two orientations allow for two chiral supersymmetries, so these theories are invariant under 10-dimensional ($D=10$) $N=2$ supersymmetry. In the type IIa theory the two supercharges have opposite chirality and so the theory has no net chirality and is not of much physical interest. Its low-energy effective point field theory limit is $D=10$, $N=2$ non-chiral supergravity, which is the theory that can be thought of as the trivial reduction of $D=11$ supergravity. The type IIb theory is more interesting because it has supercharges of the same chirality. Its low-energy point field theory limit is the chiral $D=10$, $N=2$ supergravity theory (which was actually constructed this way). This theory is remarkable in being chiral and yet not having any gravitational anomalies[16].

Unfortunately, the type IIb theory does not have any Yang-Mills gauge invariance and so it seems unlikely that interesting chiral compactified theories of this type exist.

Recently a third kind of superstring theory has been discovered, called the Heterotic string[17]. It is based on closed strings only, although it carries a Yang-Mills gauge group and has $N=1$ supersymmetry. The way the gauge quantum numbers are incorporated is very different from the method used in the type I theories. Instead of the Yang-Mills charges residing at the ends of the string there is a charge density along the string. This construction is closely related to the representation theory of Kac-Moody algebras, which has been developed over the last few years by mathematicians and physicists interested in string theories. It turns out that the only Yang-Mills groups that can be incorporated in this kind of theory are SO(32) and $E_8 \times E_8$. The occurrence of just these groups is related to the fact that they are associated with the only even self-dual 16-dimensional lattices. The construction of the heterotic string theory involves some aspects of the original 26-dimensional bosonic string theory with 16 of the dimensions being the maximal torus of one or other of the two groups (see also refs 27-29). This leaves 10 space-time dimensions. In the heterotic string theory, the gauge coupling constant and the gravitational coupling constant are related by $\kappa = \text{constant} \times g/(T)^{1/2}$.

Superstring field theory

In this section I will describe the formulation of a superstring field theory which provides a framework for describing the interactions between strings in the language of quantum field theory.

At present, the field theory of strings does not have formulation based on a geometrical principle. We are restricted to describing the theories in a special parametrization of the world-sheet swept out as the string moves, known as the light-cone parametrization. This is not very satisfying for a theory which includes gravity. Nevertheless, this field theory is convenient for perturbative calculations around flat space-time and illustrates many novel features of string theories. The open-string fields, $\Phi[x(\sigma), \theta(\sigma), \tilde{\theta}(\sigma)]$, and the closed-string fields, $\Psi[x(\sigma), \theta(\sigma), \tilde{\theta}(\sigma)]$, are functionals of the string configurations. In other words, they are functions of the superspace coordinates of the whole string and are thus generalizations of conventional point fields. The superspace coordinates in the light-cone parametrization are functions of the parameter σ which labels the points on a string and consist of the transverse positions $x^i(\sigma)$ (in ten dimensions there are eight dimensions transverse to the two light-cone directions $x^{\pm} = (x^0 \pm x^9)/\sqrt{2}$, so $i \pm 1, 2, \ldots, 8$), two grassmann spinors $\theta(\sigma)$ and $\tilde{\theta}(\sigma)$ (which are the anticommuting superspace partners of x^i) and the momentum component p^+ (which is independent of σ in this parametrization). The fields Φ and Ψ can be thought of as describing an infinite set of point fields, one for each mode of excitation of the string. The zero modes are just the usual massless fields of super-Yang-Mills and supergravity, respectively. The open-string field Φ carries the internal quantum numbers of the gauge group G (either SO(n) or USp(n)) and is therefore a matrix in the fundamental representation of the group whereas the closed-string field Ψ is a group singlet, carrying no charge.

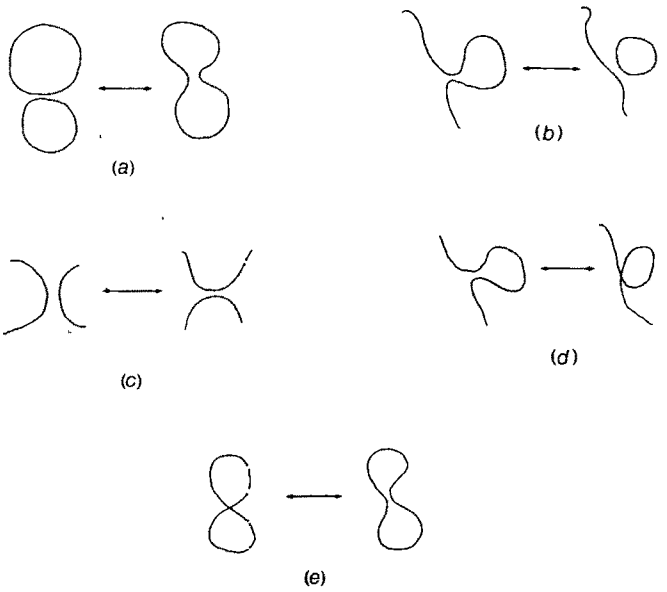


Fig. 2 The 'gravitational' interactions of strength κ. *a*, The coupling between three closed strings that contains the usual interaction between three massless gravitons. *b*–*e*, Other interactions that are locally the same as *a* at the point where the strings touch

One advantage of the light-cone parametrization is that the theory is formulated entirely in terms of a Hilbert space of physical transverse states without any redundant auxiliary or gauge degrees of freedom. However, the special parametrization destroys the manifest Poincaré invariance of the theory by picking out two directions along the light-cone in a special way. As a result, the full super-Poincaré invariance of the interacting theory is represented non-linearly on the fields. Some of the generators develop interaction terms that are cubic or of higher order in the fields. In fact, imposing the requirement that the hamiltonian of the interacting theory be invariant under super-Poincaré transformations determines it. In this manner, we have determined the superstring interaction hamiltonian which turns out to only involve local interactions only. These fall into two classes[18,19].

Class 1. 'Yang-Mills Interactions.' These are interactions with a coupling constant g in which two ends of a string join or, conversely, a string breaks to form two new endpoints. This gives rise to the term in the interaction hamiltonian, referred to earlier (pictured in Fig. 1*a* and cubic in the string fields); that is, it describes the breaking of one string into two or the joining of two strings into one. Exactly the same interaction is also responsible for the transition between open and closed strings pictured in Fig. 1*b* and mentioned above. The full expressions for these terms in the hamiltonian are given in ref. 18 as functional integrals over the string light-cone superspace. The fact that these two terms involve the same local interaction is very important for causality, because the two processes are indistinguishable near the joining point, differing only in the string boundary conditions far away from that point. These interactions generalize the usual cubic Yang-Mills coupling of point field theory. The zero-mode piece in the interaction of Fig. 1*a* is precisely the cubic super-Yang-Mills coupling.

Class 2. 'Gravitational Interactions.' These are interactions of strnegth κ in which two internal points touch and string pieces are swapped between incoming strings. An example of an interaction term of this type is shown in Fig. 2*a*, where two closed strings join to form one closed string or, conversely, one closed string splits into two. The zero-mode piece of the closed-string field is just the point superfield of supergravity and Fig. 2*a* includes the familiar coupling between three gravitons given by

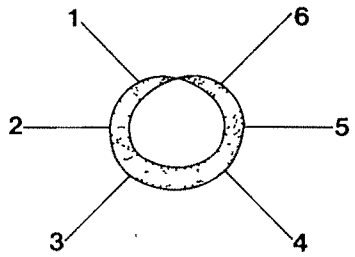


Fig. 3 The anomalous planar open-string hexagon diagram. A contribution to the anomaly in the Yang-Mills invariance

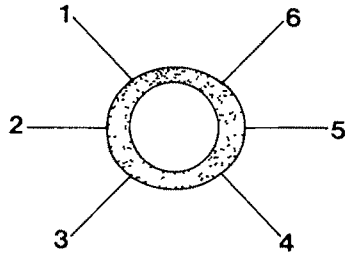


Fig. 4 One of the 32 non-orientable anomalous open-string hexagon diagrams

the Einstein-Hilbert action (as well as the infinite number of other interactions involving the excited modes). For the type II theories, the interaction of Fig. 2*a* is the only term in the interaction hamiltonian. The type I theories, which describe unoriented strings, involve five terms with this kind of interaction, shown in Fig. 2.

The heterotic string theory describes the dynamics of an oriented closed string field. Just as with the type II theories, there is only one term in the interaction hamiltonian (Fig. 2*a*). This single interaction term contains both the cubic super-Yang-Mills coupling as well as the cubic supergravity coupling

There are no higher-order contact interactions. This is quite different from point field theories like Yang-Mills or general relativity in which, for example, there are vertices which couple arbitrarily many graviton fields. These contact interactions emerge in a string theory as low-energy effective vertices resulting from the exchange of the massive string states. This is analogous to the way in which the four-fermi model of the weak interactions is now understood as a low-energy approximation to the Glashow-Weinberg-Salam theory, which involves massive W or Z boson exchange. The short-distance behaviour of superstring theories is therefore radically different from that of point field theories of gravity, as the massive modes are excited at length scales around $(T)^{-1/2}$.

These interactions, together with the free hamiltonian, lead to a set of perturbation theory rules for strings. Perturbation theory diagrams are constructed by tying together interaction vertices and free propagators to form trees and loop diagrams analogous to Feynman diagrams in point field theories.

Superstring anomaly cancellations

In earlier work we had studied one-loop perturbation theory diagrams with four external ground-state open-string particles (Yang-Mills gauge particles or their supersymmetric fermion partners). We found that, to this order, the type II theories are finite[1], unlike 10-dimensional point field theories such as type II supergravity, which diverges badly at one loop. This illustrated the possibility that superstring theory may be quantum mechanically consistent. The finiteness results from a conspiracy of divergence cancellations between the infinite numbers of supersymmetric fermion and boson states circulating around the loop. We also found that type I theories with a gauge group $G = SO(n)$ or $USp(n)$ (with general n) have a divergence (which might be eliminated by a redefinition of T). A result of the recent work to be described below is that the SO(32) type I theory is finite at one loop. These properties contrast with those of supersymmetric point field theories which, though less divergent than non-supersymmetric ones, are nevertheless badly divergent in 10 dimensions.

Recently we investigated the possible anomalies that can arise in chiral string theories in a manner analogous to the occurrence of anomalies in chiral point field theories. In 10 dimensions, an anomaly in the conservation of the Yang-Mills charge is associated with a non-vanishing coupling of the longitudinal mode of a gauge particle to five physical, transverse gauge particles in a Feynman loop diagram. This hexagon diagram is the analogue of the familiar triangle diagram associated with anomalies in four-dimensional gauge theories.

Although it should be possible to calculate the anomaly in the light-cone formalism described earlier, it is easier to use a covariant formalism based on the old 'spinning string' theory. This is not initially 10-dimensionally supersymmetric, but can be made so by projection onto a suitable subspace of the full Hilbert space. The anomalous hexagon diagrams fall into three classes: (1) The first class contains only the planar diagram shown in Fig. 3. The internal propagators in the diagram are drawn as stippled strips to emphasize that they are complete string propagators, whereas the external particles are the string ground states. The diagram is therefore an annulus formed by the world-sheet of the string circulating around the loop, with all the external particles attached to one boundary. The group theory factor associated with this diagram contains a factor of n (where the group is either $SO(n)$ or $USp(n)$) which arises because the annulus in Fig. 3 has one boundary with no external particles attached. The form of the anomaly calculated from this diagram reduces at low energy (compared with $\sqrt{T}$) to the usual point-field theory hexagon diagram result, as expected.

(2) The second class of diagrams that contribute to the one-loop Yang-Mills gauge anomaly consists of those which are non-orientable, such as the example shown in Fig. 4 which has a twist in one of the string propagators. In total there are 32 diagrams of this type which have an odd number of twists and hence world-sheets which are Möbius strips. These diagrams do not have the factor on n as they only have one boundary. In other respects they each contribute to the anomaly with the same weight as the annulus in Fig. 3 but with a relative sign that is + for the group $USp(n)$ and − for $SO(n)$.

(3) The last class of diagrams to consider consists of the diagrams with an even (but non-zero) number of twists such as Fig. 5. Remarkably, this turns out to be free of anomalies, which is apparently at odds with the corresponding low-energy limit point-field theory hexagon diagram. The resolution of this apparent contradiction is based on the fact that the open-string hexagon diagram of Fig. 5 contains closed-string bound states in the 5-6 channel. This can be seen by distorting the stippled world-sheet of Fig. 5 to look like a cylinder, as in Fig, 6, in which case the intermediate closed-string states are apparent. As the closed-string sector includes the massless supergravity multiplet, the low energy effective point field theory must include the effects resulting from the exchange of the gravity states as well as the usual hexagon loop effects. This interplay between gravity and Yang-Mills contributions is the key to understanding how the anomalies cancel.

The total Yang-Mills gauge anomaly is therefore obtained by combining the contributions from (1) and (2). The result is proportional to $(n \pm 32)$ and hence vanishes for the group SO(32). Similar arguments lead to the vanishing of gravitational anomalies (which arise from hexagon diagrams with external gravitons) for the same gauge group.

We have also reconsidered the question of infinities in scattering amplitudes. It turns out that just for the gauge group SO(32) the infinities in the four-particle scattering amplitude also cancel[19]. In fact it is probable on very general grounds that there is a connection between the absence of anomalies in chiral string theories and the cancellation of infinities in those theories to any order in perturbation theory. This is not true in conventional point field theories.

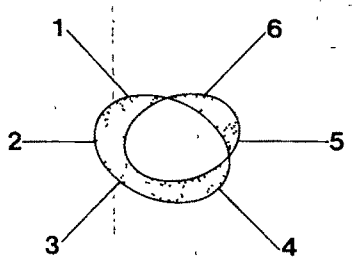


Fig. 5 A non-planar open-string hexagon diagram which turns out to be free of anomalies for any Yang-Mills gauge group

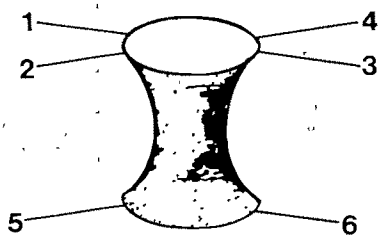


Fig. 6 Another representation for the world-sheet of the process of Fig. 5 which illustrates the closed string (gravitational) intermediate states in the channel formed by particles 5 and 6. The occurrence of gravitational states is responsible for the cancellation of the anomalies in Fig. 5.

Similar results have subsequently been found for the heterotic string theory with either gauge group SO(32) or $E_8 \times E_8$.

'Low-energy' effective point theory

The fact that all anomalies cancel for specific Yang-Mills gauge groups can be explained in the language of point field theory by considering the effective theory that emerges from the string theory at low energy. In principle the superstring field theory can be formulated exactly in terms of its massless states by integrating out the massive string-field modes in the generating functional of the theory. The resulting effective point field action, which would be expressed entirely in terms of massless fields, would be horribly complicated but could be expanded in a power series in energy/$(T)^{1/2}$ for energies $< 10^{19}$ GeV. The lowest-order terms in such an expansion give the usual point-field theory action for the $D = 10$ coupled $N = 1$ super-Yang-Mills supergravity system that had been studied previously in this 'minimal' form[20,21]. It turns out that to understand the absence of anomalies it is crucial to keep further terms in the effective action. Among the massless fields that occur in $D = 10$, $N = 1$ supergravity, there is an antisymmetric second-rank tensor, B, which plays the key role in understanding how the anomalies cancel.

The details of the anomaly cancellation in the effective point field theory are given in ref. 5 and I will simply outline the major points here. The important terms in the low-energy effective action include terms that involve the coupling of B to the other fields that are not present in the minimal action of ref. 21. These extra terms include some which spoil the Yang-Mills and the general coordinate invariances of the classical action. However, they arise in just the correct way so that they exactly compensate for the violation of these invariances by the one-loop quantum effects. The fact that this is possible depends on several remarkable identities which restrict the possible theories. For example, the cancellation of the Yang-Mills anomalies, arising from a hexagon loop with external gauge particles, restricts the possible gauge groups to ones for which

$$\mathrm{tr}\, F^6 = 1/48\, \mathrm{tr}\, F^2 (\mathrm{tr}\, F^4 - 1/300\, (\mathrm{tr}\, F^2)^2)$$

where F^{ab} is an arbitrary matrix in the adjoint representation of the group and tr denotes the trace. The gravitational anomalies arise from hexagon diagrams with external gravitons and chiral fermions circulating round the loop. These fermions are the gravitino and its spin-1/2 partner as well as the spin-1/2 matter fermions from the Yang-Mills sector (of which there are m, where m is the dimension of the Yang-Mills gauge group). For the gravitational anomalies to cancel, it is necessary for the dimension of the group to be $m = 496$. There are other conditions that arise by requiring the absence of anomalies which arise from hexagon diagrams with both external gravitons and Yang-Mills particles. The only groups that satisfy all these conditions are SO(32) and $E_8 \times E_8$. This analysis, based entirely on an effective point field theory, suggested that as well as the SO(32) superstring theory described earlier, there may be an $E_8 \times E_8$ string theory. The 'heterotic' string constructed recently[17] encompasses both SO(32) and $E_8 \times E_8$ and no others.

Towards four space-time dimensions

To make contact with physics it is important to understand the predictions of superstring theories at energies well below the Planck scale. The full superstring equations should determine the structure of space-time and hopefully lead to the collapse of the extra six spatial dimensions together with a breakdown of the very large gauge group to a subgroup which might be one of the usual GUT groups or directly to the 'standard model' with symmetry $SU(3) \times SU(2) \times U(1)$. The structure of these equations is far from understood but, nevertheless, certain general features can be abstracted by studying the terms in the expansion of the low-energy effective point field theory described earlier. The equations of motion based on this low energy approximation should not be used too literally because terms that have been dropped from the expansion may be as important as the terms that have been kept. However, certain pertinent features of the effective theory are probably properties of the complete theory. For example, as emphasized in ref. 22 there is an interesting topological constraint in the theory

$$\int (\mathrm{tr}\, R_{\mu\omega}\, R_{\rho\tau} - 1/30 \mathrm{tr}\, F_{\mu\omega}\, F_{\rho\sigma}) \varepsilon^{\mu\omega\rho\sigma} = 0$$

where $R^{mn}_{\mu\omega}$ is the Riemann curvature tensor (which is a matrix in the 0(9, 1) tangent-space group) and F^{ab} is the Yang-Mills strength (which is a matrix in the gauge group SO(32) or $E_8 \times E_8$). The integral is over an arbitrary four-dimensional compact submanifold of the full 10-dimensional space. This is a relation between topological properties of space-time and of the Yang-Mills field configurations which connects the breaking of the Yang-Mills and the space-time symmetries. It is another aspect of the unification of Yang-Mills and gravity implied by the theory. It is also, remarkably enough, the relation that is needed to ensure that the anomaly cancellation discovered in flat 10-dimensional space-time continues to work in an arbitrary curved space-time. In general, this relation requires that F has a non-zero expectation value in some subgroup H of SO(32) or $E_8 \times E_8$ in the curved dimensions, and this causes the symmetry to be broken to some commuting subgroup G which is then the symmetry group for the approximately four-dimensional theory. This may be a completely specified GUT in which the observed elementary particles are approximately massless relative to the natural mass scale of 10^{19} GeV. The spectrum of massless particles in the theory when some of the dimensions are curled up can be deduced from various index theorems on the internal space and so the number of families (and their quantum numbers) is determined by the topological structure of the theory.

One very general feature of this picture which distinguishes it from the usual Kaluza-Klein schemes is that the Yang-Mills group in the 10-dimensional theory can provide all the experimentally desirable internal symmetries. It may then be true that the dimensional compactification produces no extra gauge invariances which would happen, for example, if the internal space had no isometries. In most other schemes, these gauge symmetries are supposed to emerge from isometries of the compact internal space (for example, the $D = 11$ supergravity theory has no Yang-Mills gauge group and all the observed

four-dimensional Yang-Mills symmetries are supposed to emerge from isometries of the compact seven-dimensional space in that theory). Ricci-flat spaces have no isometries and are therefore natural candidates to consider for the extra dimensions.

There has been an interesting specific suggestion as to how the observed (approximately) four-dimensional physics might emerge from the 10-dimensional theory[23]. This makes use of a Ricci-flat space for the compact six dimensions. (Such Ricci-flat higher-dimensional spaces, known as 'Calabi-Yau' spaces, were conjectured to exist by Calabi and proved to exist by Yau[24].) Despite the fact that the full content of the string field equations is not yet understood, the authors of ref. 23 point to strong indications from the low-energy effective field theory that Ricci-flat internal manifolds are singled out. For example, if it is assumed that the four-dimensional theory has one surviving supersymmetry (desirable for phenomenological reasons), then the internal space has to be a Calabi-Yau space with holonomy group SU(3) (the curvature two-form lies in an SU(3) group), which is a severe restriction on the possible four-dimensional theories.

Furthermore, there are strong indications that the breaking of the big gauge symmetry of the 10-dimensional theory (SO(32) or $E_8 \times E_8$) is tied to the curvature in the compact dimensions. Starting with the $E_8 \times E_8$ theory in 10 dimensions, the Yang-Mills field strength associated with a SU(3) subgroup of one of the E_8 factors acquires an expectation value proportional to the curvature of the internal manifold. This causes a breaking of an SU(3) subgroup of that E_8 factor (E_8 contains $E_6 \times SU(3)$), resulting in a four-dimensional effective theory which is the supersymmetric GUT with symmetry group $E_6 \times E_8$. This is encouraging because E_6 has long been considered as a possible group for a GUT. The particle content of this unified theory is uniquely determined by specifying which Calabi-Yau space is used as the internal manifold, as the number of families is given by $1/2 \times$the Euler characteristic of the space. For simply-connected Calabi-Yau spaces, this turns out to be a rather large number, but there are multiply-connected spaces for which the number of families is smaller. These spaces also provide an attractive topological reason for why the GUT should break down to give low-energy symmetries which might well be realistic. The extra E_8 factor describes matter which only interacts gravitationally with the charged matter in the E_6 GUT because it is neutral under the E_6 Yang-Mills group. In fact, the existence of two types of matter which only interact via the gravitational force is a general prediction of the $E_8 \times E_8$ theory when it is compactified on any internal manifold with no isometries (so that no new gauge symmetries emerge from the compactification). This may have important implications for the 'dark matter' in the Universe[25] as well as for the so-called 'hidden sector' suggested by phenomenological super-unified theories for breaking the last supersymmetry.

At the present level of understanding, there is no theoretical reason for demanding one supersymmetry to survive the compactification to four dimensions. Another example mentioned as a possibility in ref. 23 is a theory in which the internal Calabi-Yau space has 0(6) holonomy and no supersymmetry survives the compactification. This results in the internal symmetry group $SO(10) \times E_8$ which is also an attractive grand unified group. These Ricci-flat internal manifolds have the virtue of not producing a cosmological constant from the compactification. But it is not yet clear why the usual purely four-dimensional sources for a cosmological constant should be absent; its absence in nature still remains a mystery.

Discussion

Recent developments have confirmed that superstring theories are interesting generalizations of point field theories containing gravity. Although these are still early days for understanding their full predictive content, the scheme proposed in ref. 23 (outlined in the previous section) suggests that there is a real chance of making contact with presently known phenomena as well as making predictions that should be testable. Other possible ways in which four-dimensional physics might emerge from these theories have been suggested in refs 23 and 26.

Our present formulation of these theories is probably not the most suitable for fully understanding their structure. For example, the relationship between the two possible Yang-Mills gauge groups and 10-dimensional space-time presumably has some geometric origin which has not been elucidated.

A more general question is why do string theories contain a massless spin-2 particle associated with gravity and a massless spin-1 particle associated with Yang-Mills theory at all? The only gauge principles used in their construction involve invariances of the two-dimensional world-sheet swept out as the string moves. String theories are not formulated in terms of any 10-dimensional gauge principle, and yet, at energy scales $< (T)^{1/2}$, they embody the principle of equivalence as well as Yang-Mills gauge invariance. Perhaps there is some generalization of the equivalence principle that gives a geometric understanding of the superstring field theory and that reduces to the usual principle at low energies. Such a generalization might be an important element in understanding the structure and hence the predictions of the theory.

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1 Green, M B & Schwarz, J. H *Nucl Phys* **B181**, 502-530 (1981), **B198**, 252-268 (1982), **B198**, 441-461 (1982), *Phys Lett* **109B**, 444-448 (1982)
2 Green, M B, Schwarz, J. H & Brink, L *Nucl. Phys* **B198**, 474-492 (1982)
3 Schwarz, J H *Phys Rep* **89**, 223-322 (1982)
4 Green, M B *Surv High Energy Phys* **3**, 127 (1983)
5 Green, M B & Schwarz, J H *Phys Lett* **149B**, 117-122 (1984)
6 Jacob, M, ed *Dual Theory* (North-Holland, Amsterdam, 1974)
7 Scherk, J *Rev mod Phys* **47**, 123 (1975)
8 Nambu, Y *Copenhagen Symp*, unpublished (1970)
9 Hara, O *Prog theor Phys* **46**, 1549 (1971)
10 Goto, T *Prog theor Phys* **46**, 1560 (1971)
11 Green, M B & Schwarz, J H *Phys Lett* **136B**, 367-370 (1984), *Nucl Phys* **B243**, 285-306 (1984)
12 Kaluza, Th *Sber preuss Akad Wiss* **K1**, 966 (1921)
13 Klein, O *Z Phys* **37**, 895 (1926)
14. Witten, E, Ranjbas-Daemi, S, Salam, A & Strathdee, J *Nucl Phys* **B214**, 491 (1983)
15 Witten, E in *Proceedings of 1983 Shelter Island II Conference* (in the press)
16. Alvarez-Gaume, L & Witten, E *Nucl. Phys* **B234**, 269 (1984)
17 Gross, D, Harvey, J Martinec, E & Rohm, R *Phys Rev Lett* **54**, 502-508 (1984)
18 Green, M B & Schwarz, J H *Nucl Phys* **B243**, 475 (1984)
19 Green, M B & Schwarz, J H *Phys Lett* **B151**, 21-25 (1985)
20 Bergshoeff, M E, B de Wit, de Roo & van Nieuwenhuisen, P *Nucl Phys* **B195**, 97-137 (1982)
21 Chapline, G & Manton, N S *Phys Lett* **120B**, 105-129 (1983)
22 Witten, E *Phys Lett* **149B**, 351-356 (1984)
23 Candelas, P, Horowitz, G T, Strominger, A & Witten, E *Nucl Phys* (in the press)
24 Yau, S-T *Proc natn Acad Sci USA* **74**, 1798 (1977)
25 Kolb, B, Seckel, D & Turner, M *Nature* **314**, 415-419 (1985)
26 Randjbar-Daemi, S, Salam, A, Sezgin, E & Strathdee, J *Phys Lett* (in the press)
27 Frenkel, I B & Kac, V *Inv Math* **62**, 23 (1980)
28 Goddard, P & Olive, D I *Proc Berkeley Workshop* (in the press)
29 Freund, P G O *Phys Lett* **B151**, 387 (1985)

The shadow world of superstring theories

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Recent attempts to construct a superstring theory that unifies all the interactions of nature, including gravity, in a finite anomaly-free quantum theory have led to the speculation that there may exist another form of matter ('shadow matter') in the Universe, which only interacts with 'ordinary matter' (for example, quarks, leptons) through gravity or gravitational-strength interactions. The existence of shadow matter would have many astrophysical and cosmological implications, some of which are discussed here.

SUPERSTRING unified theories seem to offer the possibility of constructing a consistent quantum theory that unifies all interactions, including gravity[1-3]. In fact, it has been speculated that superstrings are the only possibility for such a unification. The low-energy limit of a string theory ($E^2 \ll$ string tension $= m_{pl}^2$, where $m_{pl} = 1.22 \times 10^{19}$ GeV is the Planck mass) is an ordinary quantum field theory (QFT). Because string theories are formulated in 10 dimensions, the effective QFT we see (and feel) is the low-energy limit of a compactified string theory. Green and Schwarz[4] have shown that the only theories that are anomaly-free and finite are those based on the gauge groups SO(32) or $E_8 \times E_8$ and have constructed a type I superstring theory based on SO(32). Recently, Gross *et al.*[5] constructed a heterotic string theory based on either $E_8 \times E_8$ or SO(32). It has been speculated that if the gauge group is $E_8 \times E_8$, the $E_8 \leftrightarrow E_8$ symmetry may persist even in the dimensionally-reduced theory[4,5]. If this is the case, then there would be two forms of matter: ordinary matter, whose interactions would be described by E_8, and shadow matter, whose interactions would be described by E_8; these two kinds of matter would only interact through gravitational-strength interactions. We consider here some of the possible astrophysical and cosmological implications of 'generic' shadow matter; that is, matter which only interacts gravitationally with ordinary matter, and which may or may not be identical in its properties to ordinary matter. It should be stressed that although our interest in shadow matter was stimulated by recent developments in superstring theories, our discussions apply to any theory that predicts the existence of 'shadow matter' (that is, matter which only interacts gravitationally with ordinary matter). In fact, there are a variety of theories (for example, those with so-called 'hidden' sectors[6]) that predict shadow matter.

Shadow matter

We will consider several possible realizations for the shadow world. The first realization we will explore is that the shadow world exactly 'mirrors' the ordinary world. By this, we mean that both the microphysics of the shadow world (for example, symmetry breaking pattern, particle spectrum and masses) and macrophysics (for example, photon temperatures) are identical. After the Planck epoch (age of the Universe $t \leq 10^{-43}$ s, temperature of the Universe $T \geq 10^{19}$ GeV) we can be sure that the interaction between shadow and ordinary matter is unimportant on the microscopic level, because the rate for gravitational-strength interactions is much less than the expansion rate of the Universe. So although interactions among ordinary and shadow particles themselves will keep each separately in thermal equilibrium, the two worlds will not feel each other's presence on the microscopic level. If the two components are initially well mixed, they will remain well mixed until non-gravitational forces become important on macroscopic scales. In the standard cosmology, this does not occur until the later stages of galaxy formation. During the early stages of structure formation ($t \geq 10^{10}$ s, $T \leq 10$ eV), small density inhomogeneities grow via the gravitational (or Jeans) instability. When the density contrast $\delta\rho/\rho$ begins to go non-linear (redshifts ≤ 30), non-gravitational forces begin to play an increasingly important role and these forces will act separately on ordinary and shadow matter.

Structure formation which proceeds through the fragmentation of larger objects into smaller ones through hydrodynamic or thermodynamic instabilities will lead to the segregation of ordinary and shadow matter because of the random nature of the instabilities which act independently on the two components. In the hot dark matter or 'pancake' picture[7] large structures such as superclusters form and then fragment into galaxies through such instabilities, and so we would expect a segregation of ordinary and shadow matter on the scale of galaxies. In this case we expect to find some galaxies that are predominantly ordinary matter and others that are predominantly shadow matter, together in clusters of galaxies. If galaxies do not form from the fragmentation of a larger object, as in the cold dark matter or hierarchical picture[8], galaxies should contain equal amounts of ordinary and shadow material. However, objects that form via instabilities within galaxies, for example, stars, will have ordinary/shadow segregation. Therefore, it is reasonable to expect that even if the disk of a galaxy contains equal amounts of ordinary and shadow matter, there may be local segregation of the two components (perhaps on scales larger than our Solar System). We note that a roughly equal component of shadow matter in the disk of our Galaxy would explain one of the several dark matter problems: that the gravitational mass of the disk (as inferred from dynamics) seems to be about twice that of the material we can see or detect (for example, stars, white dwarfs, gas, dust)[9]. One might also expect some binary systems comprised of an ordinary star and a shadow star. Such a system would manifest itself as an isolated star with a periodic proper motion. In fact there are nearby stars (at distances <5 pc) which are suspected of having invisible companions[10]. Of course, there are less exotic explanations for these invisible companions; they could be neutron stars, black holes, black dwarfs or planetary-size objects.

It is possible that our Solar System formed from nebulae containing ordinary and shadow matter (although if the initial collapse of the protostellar nebulae were triggered by a shock wave in the ordinary matter, only the ordinary matter would have responded and collapsed). The shadow matter present would have formed into separate objects. However, with the possible unlikely exception of Nemesis (the death star[11,12]), we can be confident that there are no unseen planet-sized (or larger) objects in our Solar System.

Shadow matter in the Solar System

Based on the previous discussion, it seems unlikely that the Solar System would contain a significant amount of shadow matter. Those considerations aside, what can one directly infer about the amount of shadow material in the Earth or in the Sun? Material in the Earth is supported against gravity by atomic degeneracy pressure. The same would be true of shadow material in the Earth. It would settle at the centre of the Earth (any initial

motion relative to the centre would be damped by tidal dissipation) and be distributed with an approximately constant density of the order of 10 g cm^{-3}. The mass of the Earth derived from the motion of its many satellites, $M_{grav} = 4\pi \int (\rho_S + \rho) r^2 \, dr = M_S + M$, and the mass derived based on seismic determinations of ρ, $M_{seismic} = 4\pi \int \rho r^2 \, dr = M$, are consistent at about the 10% level[13]. (Here ρ and ρ_S refer to the density of matter and shadow matter, respectively.) This means that the amount of shadow material in the Earth must be significantly less than that of ordinary material.

Next, we consider the Sun. If the amount of shadow and ordinary material were equal, then the Sun would be simultaneously a star and a shadow star, each burning its own kind of hydrogen to helium. However, as we shall see, the Sun would seem very different. If the shadow matter in the Sun were distributed identically to the ordinary matter, then the equations of stellar structure for each component are identical, and equivalent to the usual equations with G replaced by $2G_N$ (G_N = the gravitational constant, whose value is 6.67×10^{-8} cm^3 g^{-1} s^{-2}) and the usual mass variable $M(r)$ (= mass interior to radial coordinate r) accounting for either the ordinary or shadow mass only (so that $M_{total}(r) = 2M(r)$). Using the standard $n = 3$ polytrope approximation to the Sun[14], it follows that the luminosity $\mathcal{L}$, rate of release of nuclear energy Q, central temperature T_c and radius R are given by

$$\mathcal{L} \propto \mu^7 (GM)^5 G^2 T_c^{1/2}$$

$$Q \propto \mu^{-3} (GM)^{-1} G^{-2} T_c^{p+3}$$

$$T_c \propto \mu^{10/(p+25)} (GM)^{6/(p+25)} G^{4/(p+25)}$$

$$R \propto \mu (GM) T_c^{-1}$$

where μ is the average molecular mass per particle and $p \simeq 4$ accounts for the approximate temperature dependence of the nuclear reactions responsible for energy generation in the Sun. (Note that the central temperature is determined by the equilibrium condition: $\mathcal{L} = Q$.)

A solar mass star composed of equal quantities of ordinary and shadow matter corresponds to $G = 2G_N$ and $M = M_S = M_\odot/2$. The $n = 3$ polytrope model of such an object predicts: $\mathcal{L} \simeq 5 \, \mathcal{L}_0$, $T_c \simeq 1.5 \, T_{c0}$ and $R \simeq 0.65 R_0$, where 'subscript zero' indicates the value of that quantity for a standard solar mass star ($M = M_\odot$, $G = G_N$) with the same chemical composition. Such a star would be easily distinguishable from 'our Sun' by its luminosity, size and solar neutrino flux (the flux of ^{8}B neutrinos detected by the Davis experiment is very temperature dependent, neutrino flux $\propto T_c^q$, $q \simeq 13$; ref. 15). Although $\mathcal{L}$, T_c and R are all dependent on the chemical composition, it is not possible to adjust μ to obtain a model which resembles 'our Sun'. Ulrich[16] and Mikkelsen and Newman[17] have constructed numerical models of the Sun where they fixed GM but allowed G to differ from G_N; from such models they concluded that G/G_N must be in the range 0.6–1.5. In passing, we note that the Chandrasekhar mass ($\equiv M + M_S$) for a star containing equal amounts of ordinary and shadow matter is smaller than the usual value by a factor of $\sqrt{2}$.

If there is only a small amount of shadow matter in the Sun ($M_S \ll M_\odot$), the shadow material will sit at the centre of the Sun and will be supported by shadow electron degeneracy pressure. The size and central density of the shadow ball at the core of the Sun will be

$$R_S \simeq 0.2 \, R_\odot (M_S/M_\odot)^{1/6}$$

$$\rho_S \simeq 2{,}000 \text{ g cm}^{-3} \, (M_S/M_\odot)^{1/2}$$

As the shadow matter would be concentrated in the core of the Sun ($r \lesssim 0.1 \, R_\odot$), it seems more reasonable to take GM fixed and $G \to G_N(1 + \rho_s/\rho_c)$, rather than $G \to G_N(1 + M_S/M_\odot)$, to estimate the effect of the shadow matter on the central temperature, etc. Using the $n = 3$ polytrope model once again, it follows that the central temperature rises by an amount $\delta T_c/T_c \simeq 6(M_S/M_\odot)^{1/2}$. As mentioned earlier, the most sensitive indicator of the central temperature of the Sun is the solar flux of ^{8}B neutrinos. Taking the flux to vary as T_c^{13} and insisting that the predicted rate does not triple, say, implies that $\delta T_c/T_c \lesssim 0.09$ or $M_S/M_\odot \lesssim 0.001$.

To conclude the discussion of shadow matter in the solar neighbourhood we can say that, apart from the possibility that Nemesis is a shadow object, there can be little shadow matter in the Solar System. We again emphasize that as the shadow matter and ordinary matter could easily be segregated in the Galaxy on scales the size of the Solar System, this observation does not preclude the existence of an exactly mirror shadow world.

Primordial nucleosynthesis

Let us turn to the early Universe. The yields of big bang nucleosynthesis depend sensitively on the expansion rate of the Universe one second after the bang, when the temperature of ordinary matter was ~1 MeV[18] The expansion rate of the Universe at nucleosynthesis is related to the total energy density, $\rho_T = \rho + \rho_S$, by

$$\dot{R}/R = [(8\pi G_N/3)(\rho + \rho_S)]^{1/2}$$

where R is the cosmic scale factor. During this early epoch the Universe is radiation-dominated, with $\rho = g_*(\pi^2/30)T^4$, where g_* counts the effective number of degrees of freedom of particles with mass less than T

$$g_* = \sum_{\text{bosons}} g_B + 7/8 \sum_{\text{fermions}} g_F$$

The addition of shadow matter results in an effective g_* at the time of nucleosynthesis

$$\rho_T = g_{\text{eff}}(\pi^2/30)T^4$$

$$g_{\text{eff}} = g_* + g_{*S}(T_S/T)^4$$

where g_{*S} counts the effective number of degrees of freedom in the shadow world and T_S is the temperature of the shadow matter, which in general need not necessarily be the same as that of the ordinary matter. Increasing the expansion rate results in the production of more ^{4}He; $\Delta Y_p \simeq 0.19 \log_{10}(1 + \Delta g/g_{\text{eff}})$[18]. Here, Y_p is the mass fraction of ^{4}He synthesized and Δg is the change in g_{eff}.

Taking into account observational data (which strongly suggest $Y_p \lesssim 0.25$), uncertainty in the neutron half life and uncertainty in the calculated abundances, Yang *et al.*[19] conclude that unless the number of light ($\lesssim$ a few MeV) neutrino species $N_\nu \lesssim 4$, ^{4}He will be overproduced. In fact if Y_p were known to be $\lesssim 0.250$ (that is, to three significant figures), $N_\nu = 4$ is not quite allowed. Note that $N_\nu = 4$ corresponds to $g_* = 12.5$.

The limit on the number of neutrinos is really a limit on the total effective number of degrees of freedom, g_{eff}. For the purposes of our discussion, we will be conservative and take the primordial nucleosynthesis limit to g_{eff} to two significant figures, $g_{\text{eff}} \lesssim 13.0$. For the exact mirror shadow world ($g_{*S} = g_*$ and $T_S = T$), $g_{\text{eff}} = 25 (N_\nu = 4)$, $21.5 (N_\nu = 3)$ or $18 (N_\nu = 2)$. Thus, big-bang nucleosynthesis eliminates exact mirror symmetry, as we know that at least two of the neutrino species are light.

Macroscopic asymmetry

Having eliminated the possibility of an exact mirror shadow world, we next discuss the possibility of a macroscopic asymmetry between the two worlds with identical microphysics. First, we note that the Solar System limits discussed in the previous section still apply. However, as before, they cannot be used to set universal limits, as shadow and ordinary matter can easily be segregated on scales as large as the Solar System. We therefore return to big bang nucleosynthesis to place limits on the macroscopic asymmetry.

A quantity which specifies the macroscopic asymmetry in a very useful way is the ratio of the entropies per co-moving volume γ

$$\gamma \equiv \frac{s_S R^3}{s R^3} = \frac{g_{*S}(T_S)}{g_*(T)} \frac{T_S^3}{T^3}$$

where s (s_S) is the ordinary (shadow) entropy density, R is the cosmic scale factor and, as before, T and T_S are the temperatures of the ordinary and shadow matter, respectively. In the absence of entropy production in either world, γ remains constant. For the scenario at hand (identical microphysics), $g_* = g_{*S}$ so that γ, the ratio of the entropy of the shadow world to our world, is just $\gamma = r^3 \equiv (T_S/T)^3$. Lacking a detailed understanding of how the compactification from $d = 10$ to $d = 4$ took place, we do not know what value of γ to expect, although starting with equal amounts of entropy in the two worlds ($\gamma = 1$) would seem to be a plausible assumption. For $g_{*S} = g_*$, $\gamma = 1$ also implies that $T_S = T$.

The primordial nucleosynthesis constraint (that is, $g_{\rm eff} \leq 13.0$) implies that

$$\gamma = r^3 \leq \begin{cases} 0.55 & N_\nu = 2 \\ 0.31 & N_\nu = 3 \\ 0.09 & N_\nu = 4 \end{cases}$$

If we assume that the ordinary and shadow baryon-to-entropy ratios are identical (however, see below), this implies that ordinary baryons must outnumber shadow baryons by a modest amount

$$n_B/n_{BS} \geq \begin{cases} 1.8 & N_\nu = 2 \\ 2.0 & N_\nu = 3 \\ 11.0 & N_\nu = 4 \end{cases}$$

Baryogenesis

If the temperatures of the shadow and ordinary matter are not the same, as primordial nucleosynthesis suggests, the shadow baryon asymmetry may differ from the normal baryon asymmetry even though the microphysics is the same for both worlds. In baryogenesis scenarios, the baryon-to-entropy ratio, B, is a function of $K = (\Gamma_I/H)_{T=M_X}$, where Γ_I is the microphysical interaction rate of some baryon number violating boson X, M_X is its mass and H is the expansion rate of the Universe ($\simeq \rho_T^{1/2} m_{\rm pl}^{-1}$; ref. 20). If the shadow and ordinary microphysics are the same and the Universe is radiation-dominated at baryogenesis ($\rho_T \propto T^4 + T_S^4$), the relevant values of K for ordinary and shadow baryogenesis (K and K_S) will be

$$K \propto \frac{m_{\rm pl}}{M_X[1+r^4]^{1/2}}$$

$$K_S \propto \frac{m_{\rm pl}}{M_X[1+r^{-4}]^{1/2}}$$

where, as before, $r = T_S/T$. If no entropy is created between baryogenesis and nucleosynthesis, r must be <1 and so $K_S < K$. The baryon asymmetry decreases with increasing K (ref. 20), so the ordinary baryon asymmetry must be less than or equal to the shadow baryon asymmetry. For $K \leq 1$, the baryon asymmetry is independent of K and thus would be the same for ordinary and shadow matter. For $K \geq 1$, the baryon-to-entropy ratio is roughly proportional to K^{-1}, and the ratio of shadow to ordinary baryon numbers is given by

$$B_S/B \simeq [1+r^{-4}]^{1/2}/[1+r^4]^{1/2} = r^{-2}$$

The number density of shadow baryons, n_{BS}, is related to the number density of ordinary baryons n_B by

$$\frac{n_{BS}}{n_B} = \frac{B_S}{B}\frac{T_S^3}{T}$$

$$= r^3 \quad (K, K_S < 1)$$

$$= r \quad (K, K_S > 1)$$

As we know that $r < 1$ from nucleosynthesis, it is difficult to imagine a scenario where shadow baryons dominate ordinary baryons (by number).

Double-bubble inflation

We have shown that γ must be <1. Even if γ were ≥ 1 initially, it is possible that it was reduced exponentially by inflation[21], as we will now explain. If the two worlds are microscopically identical we would expect inflation to occur in both sectors, but not necessarily simultaneously. Remember that inflation involves a random event—the nucleation of a bubble or the formation of a fluctuation region. At the beginning of inflation the Universe is in a false vacuum state for both worlds. A bubble will nucleate (or a fluctuation region will form) for one of the worlds first, say the shadow world. As that bubble grows exponentially in physical size (that is, inflates), both T and T_S decrease exponentially and γ remains constant. When the shadow vacuum energy is converted into radiation the shadow temperature will rise to T_{RH}, and γ increases dramatically. The Universe, however, is still inflating, driven by the vacuum energy of the ordinary sector. Eventually, a bubble (or fluctuation region) forms for the ordinary world within the shadow bubble. During this second phase of inflation, the new bubble grows exponentially in size, while both T and T_S decrease exponentially. When the vacuum energy of the ordinary world is converted into radiation, the temperature of the ordinary world rises to T_{RH}, a temperature which is exponentially larger than the temperature of the shadow world. Thus, γ has been reduced to an exponentially small value by 'double-bubble inflation'. If double-bubble inflation did occur, then the shadow world is exponentially uninteresting. We note that if inflation occurs in the 10-dimensional phase, or during the compactification transition, inflation and $\gamma = 1$ are not necessarily incompatible.

Asymmetric microphysics

Now let us consider the possibility that the symmetry between the ordinary world and the shadow world is broken microscopically. A scenario has been discussed recently where the ten-dimensional string theory is compactified on a six-dimensional, Ricci-flat Calabi–Yau Manifold to the four-dimensional theory $E_8 \times E_6$ (ref. 22). In this scheme, E_6, which eventually breaks down to $SU(3) \times SU(2) \times U(1)$, is the gauge group of the ordinary world and E_8 is the gauge group of the shadow world. (Here SU(3) is the gauge group of the strong interactions (QCD), and $SU(2) \times U(1)$ is the gauge group of the electroweak interactions.)

Once again we will use the entropy ratio of the two worlds, γ, to quantify the macroscopic asymmetry. However, when the microphysics of the worlds is not identical, g_{*S} is not necessarily equal to g_*, and so $\gamma = 1$ does not correspond necessarily to $r = T_S/T = 1$. Nevertheless, the entropy ratio is somewhat more useful than the temperature ratio in that it remains constant in the absence of entropy production, whereas the temperature ratio varies whenever g_{*S}/g_* does

$$r = (T_S/T) = \gamma^{1/3}(g_*/g_{*S})^{1/3}$$

We note that when the microphysics of the shadow world is not identical to that of the ordinary world, there are additional means (other than inflation) for changing the entropy ratio γ. They include the very out-of-equilibrium decay of a massive particle species[23–25] or a phase transition that is only mildly inflationary (for example, an entropy increase of $<10^6$ during the $SU(2) \times U(1) \rightarrow U(1)$ transition).

In the case of asymmetric microphysics, the very stringent nucleosynthesis bound can be evaded even if the two worlds have identical initial entropies. Recall that nucleosynthesis bounds $g_{\rm eff}$ to be ≤ 13. If we assume that the expansion of the Universe has been isentropic since the beginning, then $g_{\rm eff}$ is given by

$$g_{\rm eff} = g_*(1 + \gamma^{4/3}(g_*/g_{*S})^{1/3}_{\rm BBN})$$

where BBN denotes the value at big-bang nucleosynthesis, that is, when the temperature of the ordinary matter is O(1 MeV). The primordial nucleosynthesis constraint $g_{\rm eff} \leq 13.0$ results in a lower bound on $(g_{*S})_{\rm BBN}$

$$(g_{*S})_{\rm BBN} \geq \gamma^4 \begin{cases} 103 & N_\nu = 2 \\ 1{,}172 & N_\nu = 3 \\ 1.95 \times 10^5 & N_\nu = 4 \end{cases}$$

If the E_8 of the shadow world remains unbroken and the representational content of the shadow world is, as has been suggested, a single $N=1$ gauge supermultiplet, then $g_{*S}=930$ Of course, if γ is <1 (macroscopic asymmetry), the constraint could also be satisfied.

If one writes this constraint in terms of the initial temperature ratio $r_i=(T_S/T)_i$ instead of the entropy ratio γ, the constraint becomes an upper bound on g_{*S}

$$(g_{*S})_i(g_{*S})_{BBN}^{-1/4}\leq(g_*)_i r_i^{-3}(13-g_{*BBN})^{3/4}g_{*BBN}^{-1}$$

where i denotes the value of a quantity at the initial epoch and BBN the value at the epoch of primordial nucleosynthesis. If $(g_{*S})_i=(g_{*S})_{BBN}$, then the constraint becomes

$$(g_{*S})_{BBN}\leq(g_*)_i^{4/3}r_i^{-4}\begin{cases}0.213 & N_\nu=2\\ 0.095 & N_\nu=3\\ 0.017 & N_\nu=4\end{cases}$$

Massive shadow states

In gauge theories, the coupling strengths of particles are not constant, but instead evolve (or 'run') with energy. The evolution of the coupling constant g with energy scale μ is determined by the β-function

$$\mu(\partial g/\partial\mu)=\beta(g)$$

where $\beta(g)$ depends on the particle content of the theory. For the group E_8 with a single adjoint supermultiplet, $\beta(g)=-45g^3/4\pi^2$. If the coupling constant is g_0 at the Planck scale, at an energy scale μ it will be given by

$$g^2(\mu)=g_0^2(1+g_0^2 45\ln(\mu/m_{pl})/2\pi^2)^{-1}$$

If, for instance, $g_0=0.3$, then at $\mu\simeq 10^{-2}\,m_{pl}$ the coupling constant will be of the order of unity. When the gauge coupling reaches order unity in a non-abelian theory, the theory can become confining, that is, the only finite energy states in the theory are bound objects which are neutral under the gauge group, and non-abelian charge is 'confined' to these states. This phenomenon occurs in QCD at an energy scale $\Lambda\simeq O(1\text{ GeV})$: quarks become confined in colour neutral objects, hadrons. The mass scale for the bound states is the energy scale at which the gauge coupling becomes order unity. However, there can be bound states which are significantly lighter (in fact, massless) for example, Nambu-Goldstone bosons, or states which are protected from getting mass by another symmetry. The lightness of the pion (140 MeV) is understood in terms of its being a pseudo Nambu-Goldstone boson.

If the shadow group (or some non-abelian subgroup of it) remains unbroken, some coupling may become strong, say at an energy scale Λ_S, and the shadow sector may become confining. If this does occur, then the typical mass of the physical states would be Λ_S, with the possible exception of massless Nambu-Goldstone particles. In this section we will discuss the various cosmological implications of massive shadow states. We first consider the case where none of the massive states are stable, then the case where at least one massive state is stable. Although our motivation for consideration of these states is the possibility that the shadow sector is confining, our arguments do not depend on the origin of mass for the shadow states.

Consider the case where all the massive confined states can decay into shadow states of zero mass. This is possible only if all conserved quantum numbers carried by the massive states are carried by a massless state into which they can decay. If there are such massless shadow states, the typical decay width for a massive state should be $\Gamma_S\simeq\Lambda_S$. If there are no such massless shadow states, the massive ones can only decay through gravitational-strength interactions to gravitons or ordinary matter. Here, we will only treat the graviton case for which the decay width is $\Gamma_S=\Lambda_S(\Lambda_S/m_{pl})^n$, where $n=4$ for a two body bound state which annihilates to gravitons.

If $\Gamma_S\simeq\Lambda_S$, the massive states decay before they become dynamically important and the story is over. If, however, at least one massive state must decay by gravitational-strength interactions to gravitons the lifetime may be sufficiently lor that the massive state(s) dominates the energy density of th Universe before they decay. When they decay into graviton the gravitons will have an energy density $\rho_g\simeq\Lambda_S T_{SD}^3$, whe T_{SD}^3 is the number density of the massive shadow particles decay. The gravitons will act as shadow matter during nucleosy thesis as they will not thermalize with ordinary matter. At th decay epoch, ordinary matter has an energy density $\rho=T_{SD}^4 r_i^-$ After decay and through nucleosynthesis ρ_g/ρ will remain co stant. Primordial nucleosynthesis requires that ρ_g/ρ be <0 (for $N_\nu=3$); using this we can obtain a bound on Λ_S. Th massive particles decay when the expansion rate of the Univer (inverse of the age of the Universe) equals their decay widt (inverse of the lifetime). Setting $H=\rho^{1/2}\,m_{pl}^{-1}\simeq T_{SD}^2 r^{-2}m_{pl}^{-1}$ equal to $\Gamma_S\simeq\Lambda_S(\Lambda_S/m_{pl})^n$, gives $\rho_g/\rho=r^4\Lambda_S/T_{SD}$ $r_i^3(\Lambda_S/m_{pl})^{(1-n)/2}$. The limit that follows from $\rho_g/\rho<0.2$ is

$$\Lambda_S\geq(5\,r_i^3)^{2/(n-1)}\,m_{pl}$$

$$\Lambda_S\geq 3r_i^2 m_{pl}\quad(n=4)$$

If $r_i=O(1)$, the theory would have to confine at a scale $\Lambda_S\approx m$ for the massive states to be able to decay early enough to avo interfering with nucleosynthesis.

Before leaving the question of decay we would like to cor ment on the possibility that the massive states decay in ordinary matter. In that case, the decays can 'heat up' th ordinary matter and increase the ordinary entropy[25]; constrain follow from nucleosynthesis[25] and baryogenesis[23,24]. Such ve stringent constraints have been derived for the gravitino, particle predicted to exist in supersymmetric theories and whic decays only via gravitational strength interactions[26,27].

Now consider the possibility that at least one massive sta is stable (or very long lived). In this case the only way suc particles can diminish in number is by annihilations. If the are massless shadow states into which the massive state ca annihilate, the annihilation cross section will be $\sigma_A\simeq\Lambda_S^{-2}$. there are no such massless shadow states, annihilation must t via gravitational-strength interactions into gravitons (or possib ordinary matter) with $\sigma_A\simeq\Lambda_S^{-2}(\Lambda_S/m_{pl})^n$, where for two-boc annihilation via graviton exchange $n=4$. If $\sigma_A\simeq\Lambda_S^{-2}$, the rel abundance of massive particles that survive annihilation h been calculated by Wolfram[28] and Steigman[29]. The remainir massive shadow states which escaped annihilation would tod contribute a fraction of the critical density

$$\Omega_S\simeq 10^{29}r_i(\Lambda_S/m_{pl})^2\quad(\sigma_A=\Lambda_S^{-2})$$

where, because of the inherent uncertainties, we have ignore factors of g_*, g_{*S}, a logarithmic correction and the dependen on the Hubble parameter. The fact that Ω_S must be less than few implies

$$(\Lambda_S/m_{pl})\leq 3\times10^{-15}r_i^{-1/2}\quad(\sigma_A=\Lambda_S^{-2})$$

If the massive states can only annihilate into gravitons, then th annihilation rate is always much less than the expansion ra and the abundance of the massive states is not significant reduced by annihilation. In this case the present density of th massive states will be

$$\Omega_S=10^{27}\gamma(\Lambda_S/m_{pl});\qquad(\sigma_A=\Lambda_S^{-2}(\Lambda_S/m_{pl})^4)$$

Requiring $\Omega_S\leq O(1)$ implies that

$$\Lambda_S/m_{pl}\leq 10^{-27}\gamma^{-1}$$

To summarize the results of this section, if the decay of th massive confined states cannot occur through strong shado forces, then for these states to avoid interfering with primordi nucleosynthesis, Λ_S/m_{pl} must be $\geq 3r_i^2$. If this inequality is n satisfied, or if some quantum number forbids decay, then th massive states must diminish in number by annihilation, avoid contributing too much mass density at the present epoc Λ_S/m_{pl} must be $<3\times10^{-15}r_i^{-1/2}$ if there are massless confine states, or Λ_S/m_{pl} must be $<10^{-27}\gamma^{-1}$ if there are no massle confined states. If one of the annihilation bounds is saturate 'shadow relics' would be the dark matter and provide $\Omega_{total}\simeq 1$.

Conclusion

The effect of shadow matter is hard to detect in everyday life; the reader could be living in the middle of a shadow mountain or at the bottom of a shadow ocean. But it would have many effects in the early and contemporary Universe. We have shown that an exact mirror Universe is precluded by primordial nucleosynthesis. Our constraints, however, do not eliminate the possibility that shadow matter plays an interesting role in the evolution of the Universe.

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1. Schwarz, J. *Phys. Rep.* **89C**, 223-322 (1982).
2. Brink, L. *Lectures on Superstrings* (NATO Adv. Study Inst. on Supersymmetry, Bonn, 1984).
3. Green, M. B. *Nature* **314**, 409-414 (1985).
4. Green, M. B. & Schwarz, J. H. *Phys. Lett.* **149B**, 117-122 (1984).
5. Gross, D. J., Harvey, J. A., Martinec, E. & Rohm, R. *Phys. Rev. Lett.* **54**, 503-505 (1985).
6. Polonyi, J. *Budapest preprint* KFKI-1977-93.
7. Zel'dovich, Ya. B. & Novikov, I. *Relativistic Astrophysics* Vol. 2 (University of Chicago Press, 1983).
8. Peebles, P. J. E. *The Large-Scale Structure of the Universe* (Princeton University Press, 1980).
9. Bahcall, J. N. *Astrophys. J.* **286**, 169-181 (1984).
10. Mihalas, D. & Binney, J. *Galactic Astronomy*, 45 (Freeman, San Francisco, 1981).
11. Whitmire, D. P. & Jackson, A. A. *Nature* **308**, 713-715 (1984).
12. Davis, M., Hut, P. & Muller, R. A. *Nature* **308**, 715-717 (1984).
13. Dziewonski, A. M. & Anderson, D. L. *Phys. Earth planet. Interiors* **25**, 297-306 (1981).
14. Clayton, D. D. *Principles of Stellar Evolution and Nucleosynthesis* (McGraw-Hill, New York, 1968).
15. Bahcall, J. N., Huebner, W. F., Lubow, S. H., Parker, P. D. & Ulrich, R. K. *Rev. mod. Phys.* **54**, 767-799 (1982).
16. Ulrich, R. K. *Astrophys. J.* **188**, 369-378 (1974).
17. Mikkelsen, D. R. & Newman, M. J. *Phys. Rev.* D**16**, 919-926 (1977).
18. Wagoner, R. V. *Astrophys. J.* **179**, 343-360 (1973).
19. Yang, J., Turner, M. S., Steigman, G., Schramm, D. N. & Olive, K. A. *Astrophys. J.* **281**, 493-511 (1984).
20. Kolb, E. W. & Turner, M. S. *A. Rev. nucl. part. Sci.* **33**, 645-696 (1983).
21. Linde, A. *Rep. Prog. Phys.* **47**, 925-986 (1984).
22. Candelas, P., Horowitz, G., Strominger, A. & Witten, E. *Vacuum Configurations for Superstrings* (preprint, 1984).
23. Dicus, D. A., Kolb, E. W. & Teplitz, V. L. *Astrophys. J.* **221**, 327-341 (1978).
24. Harvey, J. A., Kolb, E. W., Reiss, D. B. & Wolfram, S. *Nucl. Phys.* B**177**, 456-460 (1981).
25. Scherrer, R. J. & Turner, M. S. *Phys. Rev.* D**31**, 681-688 (1985).
26. Khlopov, M. Yu. & Linde, A. *Phys. Lett.* **138B**, 265-268 (1984).
27. Ellis, J., Kim, J. & Nanopoulos, D. *Phys. Lett.* **145B**, 181-186 (1984).
28. Wolfram, S. *Phys. Lett.* **82B**, 65-68 (1979).
29. Steigman, G. *A. Rev. nucl. part. Phys.* **29**, 313-338 (1979).

Rearrangement of nitrogen fixation genes during heterocyst differentiation in the cyanobacterium *Anabaena*

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Nitrogen fixation by the cyanobacterium Anabaena *is carried out in heterocysts, specialized, non-dividing cells which differentiate under conditions of ammonia or nitrate deprivation. In* Anabaena, *heterocyst differentiation is accompanied by rearrangement of some nitrogen fixation genes. A site-specific recombination between an 11 base-pair direct repeat sequence flanking the* nifK *and* nifD *genes removes 11 kilobases of intervening DNA, resulting in juxtaposition of the two genes and an alteration of the* nifD *protein-coding sequence.*

OVER 30 years ago, McClintock suggested that a mobile genetic element inserted next to a specific gene could alter the time at which the gene was activated during development[1]. Several examples of genome rearrangements occurring either spontaneously at low frequencies or under the control of external or developmental cues have been identified subsequently, the best example of a developmentally regulated rearrangement being that of vertebrate immunoglobulin genes[2]. Other rearrangements have been described in some detail in the lower eukaryotes trypanosomes and yeast[3,4].

Genome rearrangements, termed recombinational switching, have been described in three related prokaryotic systems: *Salmonella* flagellar antigen phase variation and the host range variation of bacteriophages Mu and P1 (refs 5, 6). In each of these systems, a section of DNA is inverted by site-specific recombination in short inverted repeat sequences flanking the section. The recombination is catalysed by a recombinase encoded by DNA adjacent to one of the inverted repeats. Inversion, and consequently expression of alternate genes, is controlled by factors such as the level of recombinase. Inversion does not seem to be regulated in response to environmental cues. Transposable elements of the TnA class encode an enzyme, the product of the *tnpR* gene, that resolves cointegrate structures by deleting a section of DNA between direct repeats[7]; *tnpR* regulates its own expression but does not respond to environmental cues. The integrated form of bacteriophage λ, however, has developmentally regulated site-specific recombination[8]. When repression is lifted in response to environmental cues, the *int* and *xis* genes are transcribed. These, together with host factors, promote site-specific recombination between direct repeats in *attL* and *attR*, resulting in excision of circular λ DNA and initiation of lytic development.

We show here two developmentally regulated rearrangements of DNA adjacent to the nitrogen fixation (*nif*) genes of *Anabaena* 7120, a filamentous cyanobacterium. One rearrangement corresponds to the excision of DNA from the chromosome, resulting in the formation of an 11-kilobase (kb) circular molecule. The other, bringing DNA to a new location adjacent to a *nif* gene, could result from a deletion, inversion or transposition. These rearrangements are specific to the differentiation of heterocysts and are triggered by the environmental cue of nitrogen deprivation.

Heterocysts

Heterocysts differentiate at regular intervals (approximately every tenth cell) along filaments of *Anabaena* deprived of ammonia or nitrate[9] and provide an anaerobic microenvironment for nitrogen fixation. The ultimate product of nitrogen

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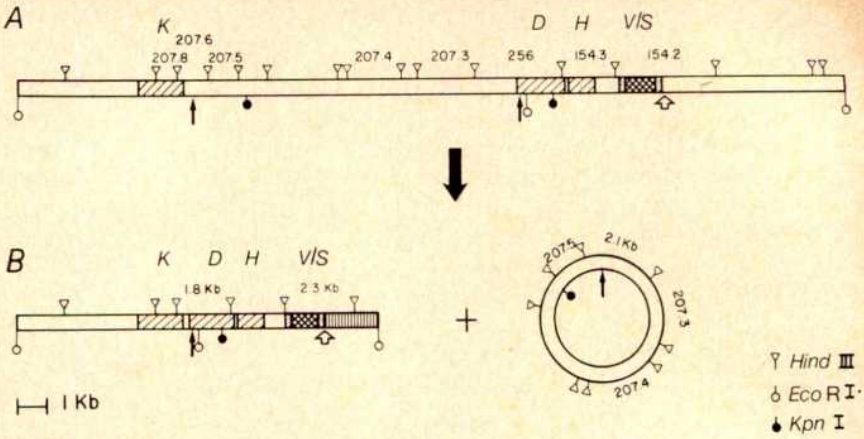


Fig. 1 Organization of nitrogen-fixation genes in *Anabaena* vegetative cell[11] and heterocyst DNA. *A*, Two contiguous *Eco*RI fragments in vegetative cell DNA containing the genes *nifK*, *nifD*, *nifH* and *nifV* or *S*. *B*, In heterocysts, the DNA is rearranged such that *nifK* and *nifD* are adjacent on the chromosome; the intervening DNA is excised from the chromosome and is found as a circle. A second rearrangement places new sequences near the *V/S* gene. The sequence originally to the right of the *V/S* gene has moved to an unknown location in heterocyst DNA. The *nifK*, *nifD* and *nifH* genes have been sequenced and their coding regions are shown[15–17]. The *nifV/S* gene has not been completely characterized, but is known to be homologous to the *nifV/S* region of *Klebsiella*. The approximate location of this homology is shown by cross-hatching[11]. *Hin*dIII restriction fragments described in the text are labelled. The position of 11-bp direct repeats involved in the excision of 11 kb between *nifK* and *nifD* are indicated by solid arrows. An open arrow indicates the approximate position of the breakpoint involved in the rearrangement near the *nifV/S* gene.

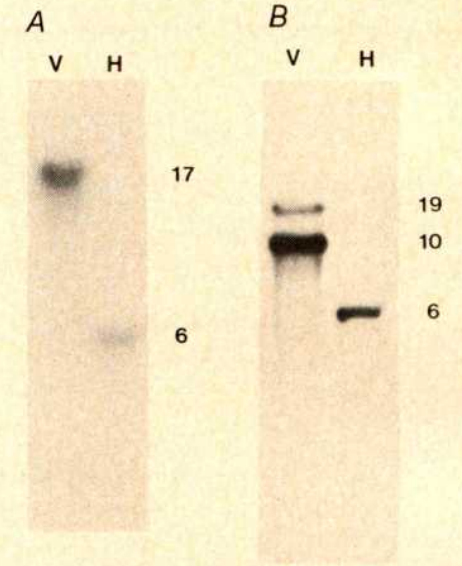


Fig. 2 Rearrangement of heterocyst DNA near the *nifK* and *nifH* genes. Vegetative cell (V) or heterocyst (H) DNA was digested with *Eco*RI and probed with *A*, 207.8 (*nifK*) or *B*, 154.3 (*nifH*). Sizes of the DNA bands are in kb.

Methods. Heterocyst purification. *Anabaena* 7120 was grown at 23–25 °C in 10 l of modified Kratz/Myers medium containing 2.5 mM $(NH_4)_2SO_4$ as described previously[13]. Filaments from a mid-log culture were collected and the cell pellet resuspended in 10 l of medium without $(NH_4)_2SO_4$ and incubated for 48–60 h. Filaments were collected by centrifugation, the cell pellets were resuspended in 100 ml of 50 mM Tris-HCl, 100 mM EDTA, *p*H 8 and lysozyme was added to 5 mg ml^{-1}. The filaments were incubated at room temperature for 60 min. The following procedures were then performed at 4 °C. The digestion mixture was diluted to 400 ml with 10 mM Tris-HCl, 10 mM EDTA, *p*H 8 (10 mM TE) and SDS was added to 0.5%. The mixture was lightly sonicated to reduce the viscosity and heterocysts were collected by centrifugation at 3,000*g* for 5 min. Debris was removed by resuspending the heterocysts in 10 mM TE and centrifuging at 500*g* for 5 min. Preparation of heterocyst DNA. Purified heterocysts were resuspended in a 25-ml Corex centrifuge tube with 5 vol. 50 mM Tris-HCl, 50 mM EDTA, *p*H 8.0, 0.5% Sarkosyl, and 0.5% Triton X-100 to which was added an equal volume of phenol/chloroform (1:1) and an equal volume of 0.45-mm glass beads. The tube was vortexed vigorously for 5–10 min, followed by centrifugation at 12,000*g* for 10 min. The aqueous supernatant was extracted twice more with phenol/chloroform (1:1), once with chloroform and then precipitated with ethanol. The nucleic acids were resuspended in 6 ml TE to which was added 3 g CsCl. This sample was layered over 2.5 ml of 5.7 M CsCl, 100 mM EDTA and centrifuged in a SW-41 rotor for 12 h at 35,000 r.p.m. RNA pellets through the lower cushion while DNA remains at the interface. The DNA was collected and banded in a CsCl-ethidium bromide gradient, dialysed, and precipitated with ethanol. Vegetative cell DNA was isolated as described previously[11]. Restriction enzymes were used according to manufacturers' recommendations. 1 μg DNA was electrophoresed through a 0.7% agarose gel and blotted onto nitrocellulose paper. Southern blotting and hybridizations were done using standard procedures[25].

fixation, glutamine, is exported to neighbouring vegetative cells and, at the same time, carbohydrate is imported from vegetative cells to provide reductant for nitrogen fixation. Complete differentiation of a vegetative cell into a mature, nitrogen-fixing heterocyst requires ~30 h. Early in differentiation, unique glycolipid and polysaccharide components of the heterocyst envelope are synthesized. Proteases are induced which break down the phycobiliprotein light-collecting apparatus as well as enzymes that fix CO_2. The photosynthetic membranes are reorganized to contain photosystem I units only, thus producing ATP by cyclic photophosphorylation without the generation of O_2 associated with photosystem II. Nitrogenase, hydrogenase and enzymes of the oxidative pentose pathway are induced, together with glutamine synthetase and elements of the transport systems for glutamine and carbohydrate.

Complete differentiation of mature heterocysts is not essential for induction of nitrogenase in *Anabaena* if anaerobiosis is established in another way. This can be accomplished by removal of combined nitrogen, addition of a herbicide to inhibit O_2 evolution by photosystem II, and bubbling the culture with argon. In these conditions, differentiation of *Anabaena* is arrested at the proheterocyst stage[10–13]. Admission of O_2 to such an induced culture results in rapid inactivation of nitrogenase and disappearance of *nif* gene transcripts[12].

Organization of nitrogen fixation genes

The genes encoding the polypeptide components of the nitrogenase complex (*nifH*, *D* and *K*) of *Anabaena* have been cloned from vegetative cell DNA, physically mapped and sequenced[11,14–17]. The physical map differs from that of the corresponding genes in the well-studied bacterium *Klebsiella pneumoniae* in an unexpected way. In *Klebsiella*, these three genes are contiguous and, based on polar insertions, are co-transcribed from a promoter next to *nifH*[18]. In *Anabaena*, the *nifH* and *nifD* genes are contiguous, but *nifK* is separated from *nifD* by ~11 kb (Fig. 1*A*; ref. 11). Another region of *Anabaena* DNA identified by homology to *Klebsiella nif* gene

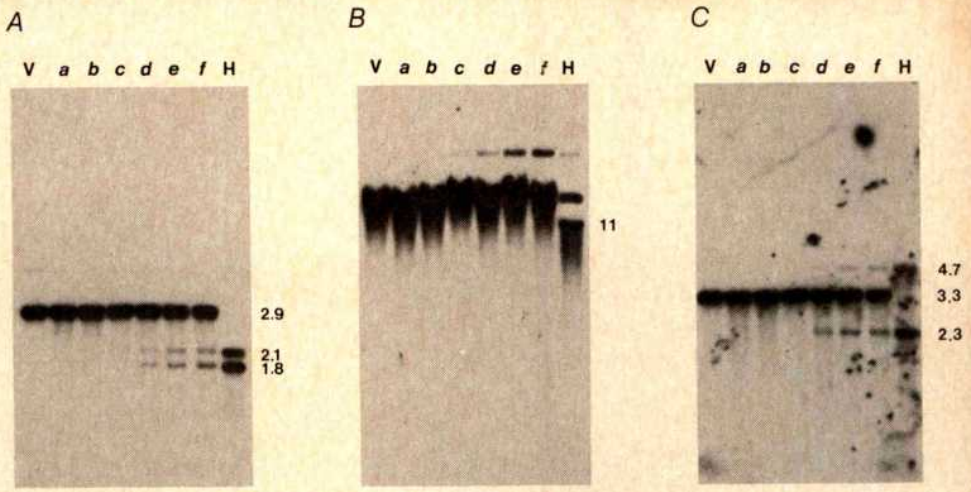


Fig. 3 Time course of DNA rearrangement and location of DNA breakpoints. *Hin*dIII-digested or undigested DNA was fractionated on 0.7% agarose gels and blotted onto nitrocellulose. *A*, *Hin*dIII-digested DNA probed with 256; *B*, undigested DNA probed with 207.3; *C*, *Hin*dIII-digested DNA probed with 154.2. (See Fig. 1 for a map of the probes.) DNA was isolated from vegetative cells (V), heterocysts (H), and at 6-h intervals during synchronous heterocyst differentiation (lanes *a*–*f*). Lanes *a*–*f* contain total DNA prepared 6, 12, 18, 24, 30 and 42 h after initiation of nitrogen starvation, respectively.

Methods. Mid-log vegetative cells from a 10-l culture containing 2.5 mM $(NH_4)_2SO_4$ were collected, washed and resuspended in 10 l of ammonia-free medium. Samples (1 l) were removed at 6-h intervals (up to 30 h) and at 42 h. The cells were collected by centrifugation, washed once with ice-cold 50 mM Tris-HCl, 50 mM EDTA, *p*H 8, and frozen in a dry ice ethanol bath. DNA was isolated from these cell pellets, which contained both vegetative and differentiating cells, as described in Fig. 2 legend. At 48 h, the cells in the last 4 l of culture were collected, heterocysts were isolated as described in Fig. 2 legend and DNA prepared; this DNA is shown in lanes H. Microscopic analysis showed proheterocysts at 18 h; 10% of the differentiated cells had polar bodies at 30 h.

probes is located ~1 kb to the right of *nifH* (Fig. 1*A*); this region contained 700 base pairs (bp) that are homologous to the *Klebsiella nifV* or *nifS* gene and has been termed *nifV/S*[11].

Transcription of the region of *Anabaena* DNA shown in Fig. 1*A* was studied by preparing RNA from cells induced for nitrogenase anaerobically. RNA from such cells hybridized with cloned DNA fragments containing *nifK*, *nifHD* and *nifV/S*, but not detectably with any of the DNA between *nifK* and *nifD*, with the possible exception of fragment 207.3 (Fig. 1*A*; hybridization to this fragment was about twice background[11]). The absence of transcripts from the intervening DNA suggests that *nifK* and *nifHD* are transcribed independently under anaerobic conditions. However, we show below that late in heterocyst differentiation the heterocyst DNA is rearranged so that the *nifD* and *nifK* genes are brought together on the chromosome by excision of the intervening DNA, allowing their coordinate transcription.

Heterocyst DNA

Heterocysts can be separated from vegetative cells because of their resistance to lysozyme and physical disruption. These physical characteristics have been an obstacle to the isolation of heterocyst DNA. A procedure originally developed for the preparation of heterocyst RNA, however, was observed to yield relatively high relative molecular mass (M_r) DNA (>20 kb) from purified heterocysts. Figure 2 shows the results of hybridizing *Eco*RI-digested vegetative cell and heterocyst DNA with two probes: 207.8, entirely within *nifK*, and 154.3, containing all of *nifH* and some 5′ flanking sequence (Fig. 1). The 17-kb band containing *nifK* in vegetative cell DNA is reduced to 6 kb in heterocyst DNA (Fig. 2*A*). Although there are other explanations, we show below that this decrease results from excision of 11 kb (see Fig. 1) from the 17-kb fragment. The probe 154.3 identifies two *Eco*RI bands in vegetative cell DNA (Fig. 2*B*). The 10.5-kb band contains part of *nifD* and a region homologous to the *nifV* or *nifS* genes of *Klebsiella*, as well as *nifH*[11]. The 19-kb band contains a second region homologous to *nifH*[11]. In heterocyst DNA, the 10.5-kb band is reduced to 6 kb (Fig. 1). The 19-kb band also appears to have disappeared, but the heterocyst DNA is sufficiently sheared before restriction endonuclease digestion such that it cannot yield 19-kb fragments with two *Eco*RI ends. Probing with the entire 19-kb *Eco*RI fragment cloned from vegetative cell DNA of other enzyme digests (for example, *Hin*dIII, *Cla*I) of heterocyst DNA has failed to detect evidence for rearrangement on either side of the second *nifH* gene (data not shown).

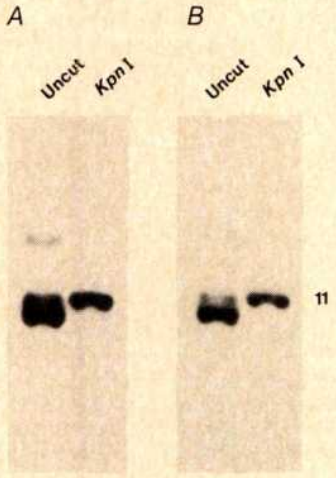


Fig. 4 Rearrangement results in the formation of an 11-kb circle. Undigested and *Kpn*I-digested heterocyst DNA was size-fractionated on a 0.7% gel, blotted onto nitrocellulose and probed with *A*, 207.4 and *B*, 207.5. Probes 207.4 and 207.5 are to the right and left, respectively, of the single *Kpn*I site found in the excised circle (Fig. 1).

Methods. Unlike the gels shown in Figs 2 and 3, the 0.7% agarose gel was run at low voltage (1.5 V cm^{-1}) in the absence of ethidium bromide for 21 h. After hybridization with probe 207.4 and exposure to film, the probe was removed by washing for 5 min in H_2O at 100 °C. Probe removal was confirmed by autoradiography and the blot was then hybridized with probe 207.5.

Heterocysts stain poorly with Feulgen's reagent; it has been suggested that they contain no DNA[9]. Thus it seemed possible that, during differentiation, the vegetative cell DNA was shattered in a manner analogous to that of the DNA in macronuclei of certain protozoa[19]. Probing with other cloned genes, however, showed no evidence of breakage in heterocyst DNA. These probes included the *Eco*RI fragment to the left of *nifK*, the *psbA* gene coding for the M_r 32,000 (32K) thylakoid membrane protein[20] and the *glnA* gene coding for glutamine synthetase[13]. For each probe, the pattern of hybridization with heterocyst DNA was identical to that of vegetative cell DNA (data not

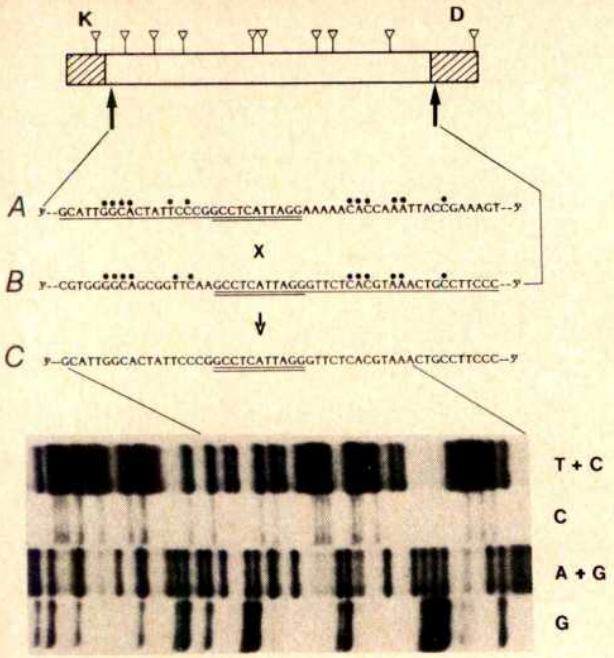


Fig. 5 DNA sequence of the fused chromosome between *nifK* and *nifD* after excision of the 11-kb fragment. The lower sequence (*C*) is that of the rearranged chromosome which contains one copy of the 11-bp direct repeat (double underline) flanked by sequences found 5′ to the *nifK* gene and within the 3′ coding region of *nifD*. The vegetative cell sequences (*A*, *B*) are shown above the rearranged sequence with the portions that are joined in the heterocyst chromosome underlined. The positions of the 11-bp direct repeats in the vegetative cell chromosome are indicated by arrows. Dots indicate partial homology in the sequences flanking the 11-bp direct repeat.

Methods. Heterocyst DNA was cloned into the vectors λgt7ara6 and λL47.1. DNA was partially digested with *Sau*3A and size-fractionated on a 0.8% low-gelling temperature agarose gel. Fragments between 8 and 20 kb were isolated and ligated into *Bam*HI-digested λL47.1 arms, packaged *in vitro*, and amplified[25]. A second library was constructed from a complete *Eco*RI digest of heterocyst DNA ligated into *Eco*RI-digested λgt7ara6 arms. This library was screened without amplification. Several clones containing the excison were obtained from the λL47.1 library using probes 207.6, 207.3 and 256. This library failed to yield clones of the rearranged chromosome. The λgt7ara6 library was screened with probes 207.8 and 154.3, yielding clones of the *Eco*RI fragments containing the *nifK* and *nifH* genes, respectively. The *Eco*RI fragments were subcloned into the plasmid vector pBR328. Breakpoints were localized in the cloned DNA by probing Southern blots of restriction digests with the known breakpoint probes 256 and 207.6. Sequence analysis was by the method of Maxam and Gilbert[26] as modified by Smith and Calvo[27].

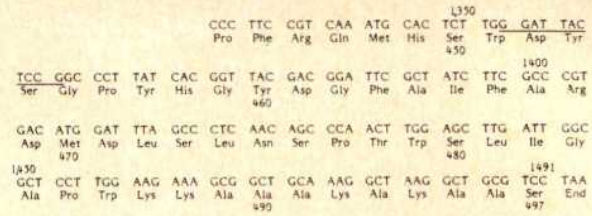


Fig. 6 Sequence of the α-subunit of *Anabaena* dinitrogenase in the rearranged *nif* region of heterocysts. The underlined nucleotide sequence is the 11-bp direct repeat (5′→3′) at the site of recombination. This corresponds to nucleotides 1,353–1,363 of the published *nifD* sequence[17] and to nucleotides 200–210 in the 5′ region of *nifK*[16]. Numbers in the figure refer to the start of the *nifD* ORF[17]. The amino acids starting with Gly 455 replace 26 amino acids predicted from the sequence of *nifD* determined on vegetative cell DNA[17]. Following the TAA stop codon there are 199 bp before the ATG that starts the *nifK* ORF.

shown). The faint banding pattern seen in ethidium bromide-stained gels of restriction digests showed no apparent differences between vegetative cell and heterocyst DNA.

As the changes shown in Fig. 2 are specific for the *nif* genes, we next asked when they occur during heterocyst differentiation. For this purpose a culture was synchronously induced aerobically and samples were removed every 6 h. DNA was isolated from total cells (10% of which were differentiating), from non-induced vegetative cells and from heterocysts separated from vegetative cells (48 h after induction), as described in Fig. 2 legend. Each DNA preparation was then digested with *Hin*dIII (or not digested), electrophoresed and hybridized with nick-translated probes. Figure 3*A* shows the *Hin*dIII-digested DNA probed with 256, which contains the right-hand breakpoint of the excised 11-kb segment (Fig. 1). Vegetative cells show a single 2.9-kb *Hin*dIII band. The major band at 2.9 kb remains at all time points because of non-differentiating vegetative cells. The two new bands in heterocysts arise from the newly fused chromosome (1.8 kb) and the joined ends of the excised circle (2.1 kb). The identity of these bands was confirmed by sequence analysis. In heterocyst DNA, the same two bands are identified by 207.6, the *Hin*dIII fragment containing the left-hand breakpoint 5′ to the *nifK* gene (data not shown).

Figure 3*B* shows undigested DNA probed with 207.3, a *Hin*dIII fragment located entirely in the excised segment (Fig. 1). The presence of multiple discrete bands in undigested DNA from heterocysts indicates that this DNA is circular. Figure 4 shows the heterocyst DNA undigested or digested with *Kpn*I, a restriction enzyme for which there is a single site in the excised segment[11]. The two probes used in Fig. 4 are located on either side of the *Kpn*I site (see Fig. 1); as they identify the same single band in the *Kpn*I-digested DNA, the multiple bands in undigested DNA must correspond to isomeric or polymeric forms of the same circular molecule. Different preparations of heterocyst DNA show different ratios of these various forms. For convenience, we shall refer to the 11-kb sequence as an 'excison'. The excison sequence is unique in *Anabaena* 7120 DNA (Figs 3*A*, 4).

The DNA samples in Fig. 3*C* were digested with *Hin*dIII and probed with 154.2, which contains the breakpoint to the right of *nifV/S* (Fig. 1). Vegetative cell DNA showed the expected single 3.3-kb band, whereas in heterocyst DNA this band was replaced by two bands of 4.7 and 2.3 kb. The new genomic location of the 4.7-kb *Hin*dIII fragment is unknown. The 6-kb *Eco*RI fragment identified in heterocyst DNA by the probe 154.3 (see Fig. 2) has been cloned (see Fig. 5). This clone contains the left portion of 154.2 on a 2.3-kb *Hin*dIII fragment, showing that this fragment in heterocyst DNA contains the *nifV/S* gene. The 6-kb *Eco*RI fragment contains, at its right end, ~2 kb of DNA originally located at least 15 kb away in vegetative cell DNA (Fig. 1*B*). This was determined by using the small *Hin*dIII/*Eco*RI fragment from the right end of the 6-kb *Eco*RI fragment (Fig. 1*B*) as a probe on restriction digests of vegetative cell DNA. This fragment identified a 3.5-kb *Eco*RI fragment and a ~16-kb *Hin*dIII fragment (data not shown). Comparison of these results with the known restriction map of the *nif* region of *Anabaena*[11] indicates that the new DNA originates at least 15 kb away from the *nifV/S* gene in vegetative cell DNA.

Comparison of the three parts of Fig. 3 shows that the excision and circularization of the 11-kb segment, the fusion of *nifD* and *nifK* and the rearrangement to the right of *nifV/S* occur simultaneously within the time resolution of the experiment. The rearrangements are late events in heterocyst differentiation, as is the appearance of nitrogenase activity[9]. Early proheterocysts, identified by their morphology, are first seen at 18 h, when the rearranged DNA fragments are detected faintly. At 24 h, the rearrangements are clearly visible, corresponding to the time at which *nifH* message can first be detected (data not shown).

Sequence of *nifK/nifD* rearrangement

To elucidate the mechanism of the excision between *nifK* and *nifD*, the two fragments of heterocyst DNA containing the breakpoints (Figs 1, 3) were cloned and sequenced. Two different clones of the excison were obtained from a partial *Sau*3A digest of heterocyst DNA cloned in the vector λL47.1 (ref. 21). Both of these clones contained 11-kb inserts, which included the 2.1-kb *Hin*dIII junction fragment formed from the right portion of 207.6 and the left portion of 256 (Fig. 1). Restriction analysis showed that the two clones were circularly permuted, differing only in the *Sau*3A site originally digested. Further restriction analysis comparing the 2.1-kb junction fragment with 207.6 and 256 was used to localize the junction for sequencing.

The junction created in the chromosome by the excison was isolated from a library of *Eco*RI fragments of heterocyst DNA cloned in the vector λgt7ara6 (ref. 22). The previously determined sequence of the junction in the excised DNA was used to predict the site of the junction on the chromosome. A portion of one sequencing gel, corresponding to the fused chromosome, is shown in Fig. 5. The new fragment (Fig. 5*C*) contains *nifK* 5′ flanking sequence[16] up to the 11-bp sequence 3′-GCCTCAT-TAGG-5′ (underlined in *A*) and then it contains nucleotides within what was believed to be *nifD* coding sequence (underlined in *B*)[17]. The junction fragment from the excison contains the reciprocal sequence with one copy of the 11-bp sequence. Thus, the excision event is a conservative site-specific recombination between 11-bp direct repeats separated by 11 kb in the vegetative cell chromosome.

The nucleotide sequence of the *Anabaena nifD* coding region was determined previously using cloned vegetative cell DNA[17]. The open reading frame (ORF) predicts a polypeptide of 480 amino acids. After excision, a new ORF is created by the fusion of the *nifD* open reading frame to *nifK* 5′ flanking sequence. This new ORF codes for 497 amino acids, of which the C-terminal 43 residues replace 26 C-terminal residues of the vegetative cell ORF (Fig. 6). The new ORF predicts an amino-acid sequence for the *Anabaena* nitrogenase α-subunit that is much more homologous to the *nifD* proteins of parasponia *Rhizobium* ANU289 (ref. 23) and cowpea *Rhizobium* IRc78 (ref. 24). Before the rearrangement, 1 of 26 C-terminal residues matches between *Anabaena* and *Rhizobium*; after the rearrangement, 28 of 43 residues are identical. Thus, it seems that the 11-kb element interrupts the real *nifD* coding region in *Anabaena*. The rearranged heterocyst chromosome allows the transcription of the *nifH, D* and *K* sequences as a polycistronic message, as in *Klebsiella*. Probes for *nifH*, *nifD* and *nifK* all identify a 4.7-kb band (with smaller transcripts) in RNA isolated from cells induced for nitrogenase, both aerobically and anaerobically (data not shown).

Why rearrange?

The two rearrangements of the *Anabaena* chromosome described here are associated with the cellular differentiation of heterocysts induced by starvation for combined nitrogen. The rearrangements are quantitative, in the sense that they occur in every genome of the differentiating cells. They are late events in the differentiation; Fig. 3 shows the first evidence for rearrangement at 18 h, while the morphological changes that characterize proheterocysts are mostly complete by 12 h. Establishment of anaerobiosis may be a necessary prerequisite for the rearrangements.

We do not yet know the functional consequence of the *nifV/S* gene rearrangement. The excision resulting in fusion of the *nifK* and *nifD* genes, however, creates an operon whose 4.7-kb transcript contains sequences of the *nifH* gene as well. It may be advantageous, and perhaps even necessary, to excise the 11-kb sequence in order to express the *nif* structural genes in *Anabaena*. The function of the 11-kb sequence in vegetative cells is unknown; it does not seem to be required for the prevention of the synthesis of nitrogenase as control of transcription initiation at the *nifH* promoter is sufficient for that purpose. We considered the possibility that a functional gene was created on the 11-kb circle by fusion of the ends of the excison, but no ORF exists in 500 bp on either side of the 11-bp repeat on the circle.

Excision of the 11-kb segment involves conservative recombination between directly repeated sequences, analogous to the excision of bacteriophage λ DNA from a lysogen[8]. Extending this analogy, it is possible that the excision is controlled by regulating synthesis of the excision enzyme. The mechanism by which the gene encoding this enzyme is regulated remains unknown, as does the regulation of transcription from the *nifH* promoter.

We are not aware of any cellular phenomena among prokaryotes or eukaryotes that display the features described here for the *Anabaena nif* gene rearrangement; that is, an environmentally triggered excision resulting in operon fusion. The closest analogy is the excision of bacteriophage λ from a lysogen. In this example, however, the elaborate controls of lysogeny have been selected to benefit the virus, rather than the host. The results presented here suggest that the 11-kb sequence confers a selective advantage on its host; how it does so remains to be discovered.

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1. McClintock, B. *Cold Spring Harb. Symp. quant. Biol.* **16**, 13-47 (1951).
2. Kataoka, T., Kawakami, T., Takahashi, H. & Honjo, T. *Proc. natn. Acad. Sci. U.S.A.* **77**, 919-923 (1980).
3. Borst, P. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 622-659 (Academic, New York, 1983).
4. Haber, J. E. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 559-619 (Academic, New York, 1983).
5. Silverman, M. & Simon, M. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 537-557 (Academic, New York, 1983).
6. Toussaint, A. & Resibois, A. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 105-158 (Academic, New York, 1983).
7. Heffron, F. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 223-260 (Academic, New York, 1983).
8. Campbell, A. in *Mobile Genetic Elements* (ed. Shapiro, J. A.) 66-103 (Academic, New York, 1983).
9. Haselkorn, R. *A. Rev. Pl. Physiol.* **29**, 319-344 (1978).
10. Rippka, R. & Stanier, R. Y. *J. gen. Microbiol.* **105**, 83-94 (1978).
11. Rice, D., Mazur, B. J. & Haselkorn, R. *J. biol. Chem.* **257**, 13157-13163 (1982).
12. Haselkorn, R., Rice, D., Curtis, S. E. & Robinson, S. J. *Ann. Microbiol. Inst. Pasteur* **134B**, 181-193 (1983).
13. Tumer, N. E., Robinson, S. J. & Haselkorn, R. *Nature* **306**, 337-342 (1983).
14. Mazur, B. J., Rice, D. & Haselkorn, R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 156-160 (1980).
15. Mevarech, M., Rice, D. & Haselkorn, R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6476-6480 (1980).
16. Mazur, B. J. & Chui, C. F. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6782-6786 (1982).
17. Lammers, P. J. & Haselkorn, R. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4723-4727 (1983).
18. Ausubel, F. M. & Cannon, F. C. *Cold Spring Harb. Symp. quant. Biol.* **45**, 487-496 (1980).
19. Ammermann, D., Steinbrück, G., von Berger, L. & Henning, W. *Chromosoma* **45**, 401-429 (1974).
20. Curtis, S. E. & Haselkorn, R. *Pl. molec. Biol.* **3**, 249-258 (1984).
21. Loenen, W. A. M. & Brammar, W. J. *Gene* **20**, 249-259 (1980).
22. Davis, R. W., Botstein, D. & Roth, J. R. *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, New York, 1980).
23. Weinman, J. J., Fellows, F. F., Gresshoff, P. M., Shine, J. & Scott, K. F. *Nucleic Acids Res.* **12**, 8329-8344 (1984).
24. Yun, A. C. & Szalay, A. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7358-7362 (1984).
25. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
26. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
27. Smith, D. R. & Calvo, J. M. *Nucleic Acids Res.* **8**, 2255-2275 (1980).

Quasar 4C39.25 is not contracting

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The milli-arc second radio structure of the quasar 4C39.25 has previously been described as consisting of two components whose angular separation remained constant at ~2 marc s, whereas their relative flux densities varied with time[1–3]. This behaviour is in marked contrast to other similar sources whose radio structures expand superluminally[4]. Recently, Shaffer[5] suggested that 4C39.25 may have been contracting superluminally in the period 1979–82. Here, based on our map of this source made from VLBI observations in 1983 at λ3.6 cm, we conclude that this conjecture is not correct. We find three distinct components in the structure, two of which are separated by 2.0 marc s, whereas the third, presumably new and not previously reported, is situated between the other two. It is possible either that the third component is stationary and that its flux density has rapidly increased to render it visible, or that it has recently been ejected from the westernmost component.

Our VLBI observations of 4C39.25 at a radio wavelength of λ3.6 cm took place on 10 May 1983. We recorded data for 2 min at 30 min intervals for over 7 h. We used Mark III instrumentation[6], a synthesized bandwidth of 28 MHz, right-hand circular polarization and the following array of eight radio telescopes: 100 m, at Effelsberg, FRG; 64 m, at Robledo, Spain; 20 m, at Onsala, Sweden; 37 m, at Westford, Massachusetts, USA; 43 m, at Green Bank, West Virginia, USA; 25 m, at Fort Davis, Texas, USA; 64 m, at Goldstone, California, USA; and 40 m, at Big Pine, California, USA. Hydrogen maser frequency standards were used at each site. The data were processed at the Mark III processor of the Max-Planck-Institut für Radioastronomie in Bonn, FRG. After calibration of the visibility amplitudes[7] we produced a map of the source using standard hybrid mapping techniques. To improve the relative calibration between the antennas we used the Cornwell and Wilkinson[8] algorithm of self-calibration, which, given the large number of antennas available, worked very well. The resulting map is presented in Fig. 1.

We have labelled the components in Fig. 1 as *a*, *b* and *c*. The separation of the centre of component *c* from that of *a* is 2.0 ± 0.1 marc s; the separation of *b* from *a* is 1.4 ± 0.1 marc s. During the 1970s the structure was well characterized at λ2, λ2.8, λ3.6 and λ6 cm by two components separated by 2 marc s[1–3]. More recently, Shaffer[5] mapped the source from observations at λ2.8 and λ3.6 cm and found it to consist of two components, their angular separation significantly <2.0 marc s, the western component being elongated. As a result, he conjectured that the source may be contracting, but he also recognized that motion in a source could be mimicked by the appearance of a new component. From our result we infer that the elongation of Shaffer's western component resulted from a blending of our two components *b* and *c*, and conclude that our *a* and *c* components correspond to those observed in the 1970s, that *b* is a new component and that the source has, therefore, not contracted.

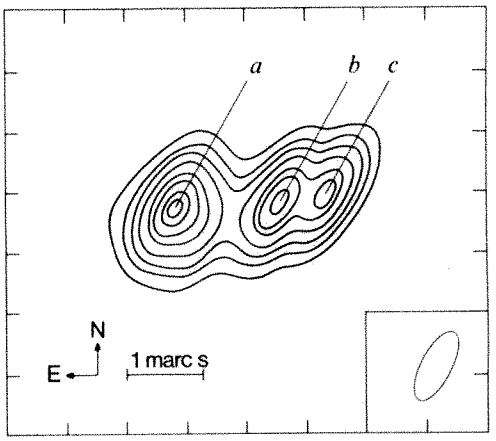


Fig. 1 Hybrid map of 4C39.25 made from λ3.6-cm data from epoch 10 May 1983. The restoring beam used is shown boxed in the lower right-hand-side corner. The contours specified are 4, 10, 18, 30, 50, 65, 85 and 95% of the peak brightness temperature.

The origin of component *b* is not clear: it could be a stationary component whose flux density has rapidly increased or it may have been ejected, say, by component *c*. Curiously, according to H. D. Aller and M. F. Aller (personal communication) the flux density of 4C39.25 has been decreasing steadily at wavelengths from λ2 to λ6 cm since 1980 with no obvious new outbursts, whereas the linear polarization at λ2 cm has increased from 1 to 2.5% since 1983. We cannot distinguish between the two proposed alternatives without the detailed analysis of other VLBI observations of comparable resolution made at different epochs. A detailed comparison of the results from our May 1983 observations, with observations at λ2.8 cm from June 1982 with similar resolution (which confirm the existence of the new *b* component) and with observations at λ6 cm from July 1982, will be presented elsewhere.

If the component *b* is stationary, 4C39.25 would surprise us again by exhibiting a milli-arc second morphology quite different from that of other radio sources whose radio spectra and variability characteristics are shared by 4C39.25. On the other hand, if component *b* has been ejected from component *c*, then its apparent motion may well be found to be superluminal. Moreover, it may overtake component *a*, as Shaffer[5] noted. Such a possibility would be rather exciting, offering a unique opportunity to test different hypotheses on the nature of the components of compact radio sources.

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1. Shaffer, D. B. *et al. Astrophys. J.* **218**, 353–360 (1977).
2. Bååth, L. B. *et al. Astr. Astrophys.* **86**, 364–372 (1980).
3. Pauliny-Toth, I. I. K. *et al. Astr. J.* **86**, 371–385 (1981).
4. Porcas, R. *Nature* **302**, 753–754 (1983).
5. Shaffer, D. B. *Proc. IAU Symp.* No. **110**, 135–136 (1984).
6. Rogers, A. E. E. *et al. Science* **219**, 51–53 (1983).
7. Marcaide, J. M. *et al. Astr. Astrophys.* **142**, 71–84 (1985).
8. Cornwell, T. J. & Wilkinson, P. N. *Mon. Not. R. astr. Soc.* **196**, 1067–1086 (1981).

The radio jet of the quasar 3C273

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Although 3C273 was was one of the first quasars to be identified[1], the extended feature 3C273A, which can be detected at radio[2,3], optical[4,5] and X-ray wavelengths[6], remains an enigma. The source is an extreme example of a one-sided radio source (3C273A has no detectable counter component[2]) and this fact, coupled with the presence of the optical emission, makes it unlikely that 3C273A is a normal (slow-moving) radio lobe. Superluminal transverse motion[7,8] at milliarc second scales shows that relativistic velocities occur within the quasar itself, 3C273B; it is an open question (see ref. 2) whether these velocities persist out to 3C273A. It has been widely suggested[9,10] that Doppler beaming causes the one-sidedness of this and similar sources by suppressing the receding half of the source, but there are no spectral lines by which the Doppler shift of 3C273A could be directly measured. Thus, any (indirect) indication of the velocity is of interest. We present here new MERLIN observations of the brightness and polarization of the radio jet of 3C273 at a resolution of 0.35 arc s. One of the most marked features of our new map, the high polarization found within the head of the source, is hard to explain. If the motion is indeed fast, then relativistic aberration should be taken into account; we suggest that this leads to a natural explanation of the high observed polarization.

The MERLIN interferometer[11] has hitherto only been able to map brightness distributions, but we have now added the facility of mapping in linear polarization (to be described more fully elsewhere). In brief, a map of brightness is produced first, using the hybrid-mapping procedure of Cornwell and Wilkinson[12], and the relative amplitudes and phases of the polarization data[13] are then used to construct the map of complex polarization $P = (Q + jU)$. The first polarization map, made from observations taken in April 1984, shows 3C273A at 1,666 MHz (λ 18 cm) with a resolution of 0.35 arc s (Fig. 1). The intensity data agree well with observations (A. R. Foley and R. J. Davis, personal communication) taken with MERLIN some three years earlier. Because MERLIN has few short-spacing baselines, it is difficult to map low-brightness regions at 18 cm, and neither Fig. 1 nor the earlier 18-cm map includes the fainter portions of 3C273A, which can be seen on the MERLIN map[2] at 73 cm, or on the Very Large Array (VLA) map[3] at 6 cm. Although the core, 3C273B, does not appear on the map, it has a flux density at this epoch of 31.0 Jy and a polarization of $1.7 \pm 0.1\%$ in position angle (p.a.) $151 \pm 8°$ (E-vector). These values have changed little since 1973 (R. J. Davis, personal communication).

The brightest component of 3C273A, labelled 2 in Fig. 1 (numbering from the outer end of the jet), has a linear polarization of $25 \pm 2\%$. The mean B-vector is at p.a. $128 \pm 3°$, that is, 86° to the main axis of the jet, here taken as 42°. In contrast, the precursor, component 1, has a low polarization, $\simeq 2 \pm 2\%$ (the uncertainty is caused by the possibility of side lobes from component 2). The magnetic field goes goes through a transition at or near component 3, and at all points further back than component 3 the field is parallel to the jet, to within a few degrees. Component 3 itself, which lies at an angle to the main axis, marks the start of the sinusoidal oscillation of the ridgeline (the 'wiggle') noted in refs 2 and 3.

In all these respects Fig. 1 shows good agreement both with the 6-cm VLA map[3] and with an unpublished VLA map at 2 cm (R. A. Perley, personal communication). It is also consistent with earlier observations[14] made with single baselines at several wavelengths. The rotation measure of component 2 is $+1.80 \pm 0.20$ rad m^{-2}, and any variations over the map seem to be $\ll 0.5$ rad m^{-2}. (The value of rotation measure corresponds to 3° of rotation at λ 18 cm, which has been allowed for in determining the magnetic position angles.) We are undertaking a full comparison of all the available radio data and will submit a more detailed discussion elsewhere; our purpose here is to discuss the unexpectedly high polarization of component 2.

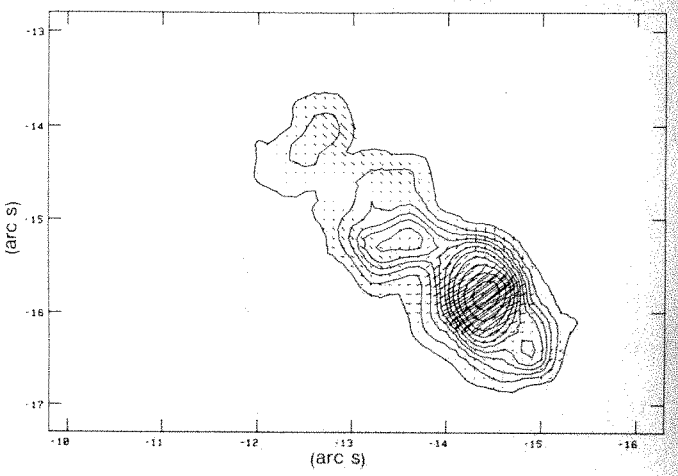


Fig. 1 MERLIN map of the radio jet in 3C273 at 1,666 MHz. The core (3C273B) is at the origin of coordinates, off the top-left of the map. The resolution is 0.35 arc s and the contours represent 0.2, 0.4, 0.6, 0.8, 1.0, 1.3, 1.6, 2.0, 2.5, 3.2, 4 and 5% of the brightness of the core, which is 31.5 Jy per beam. B-vectors of polarization (after allowing for a Faraday rotation of 3°) are superimposed on the total intensity contours. A vector 1 arc s long represents a polarized intensity of 530 mJy per beam.

Laing[15] has studied the polarization occurring in plasma that is sheared or compressed anisotropically. The apparent degree of polarization for a Laing sheet (that is, a thin sheet of plasma with the direction of magnetic field confined to the plane of the sheet) varies with aspect angle. If the spectral index is 0.7 as here, a polarization of 25% implies that θ_s, the angle between the line of sight and the normal to the sheet, is 47°. When the compression is partial and the magnetic field less well-ordered, as in a thick sheet, the aspect angle θ_s must be greater than 47°.

This argument seems to be completely at variance with the evidence of superluminal motion, which can only occur if the ejection axis is at a small angle to the line of sight. If Hubble's constant is 100 km s^{-1} Mpc^{-1}, the observed proper motion[7] of 0.76 marc s yr^{-1} at a distance of the source ($z = 0.158$) implies a transverse velocity $\beta_{obs} = 4.6$. The most probable angle to the line of sight is $\theta_0 = \tan^{-1}(1/\beta_{obs}) = 12°$, and for this angle the speed is $0.98c$, with a Lorentz factor $\gamma \simeq 5$. A velocity β_{obs} of 4.6 cannot be observed if θ_0 is 47°.

There are several possible explanations of this apparent paradox:

(1) The superluminal motion may be entirely unconnected with the jet, but we reject this hypothesis on grounds of probability.

(2) The jet axis may be bent from $\theta = 12°$ near the nucleus to 47° in the outer regions. Bends are common in jets, and a bend in position angle of 23° occurs in 3C273B[7,16], about 8 marc s from the core. This explanation therefore cannot be wholly excluded, but because position-angle bends are magnified by foreshortening, the real angle of bend is probably $\leq 5°$, whereas a bend of at least 35° would be needed to obtain $\theta_s = 47°$.

(3) Component 2 may be inclined at an angle to the axis. Hotspots set back from the head are found in other sources[17] and can be interpreted as sites where the active beam intersects the side of a pre-existing cavity[10]. In the process, an oblique shock could be set up, which would account for component 2 if the normal to the shock were more than 35° from the jet axis. For the projection of the magnetic field to appear transverse to the axis, however, the normal to the shock must lie rather exactly in the plane of the jet axis and the line of sight. It is observed in other sources that the projected magnetic fields are invariably aligned parallel with the boundaries of emission; when seen at low resolution, the resultant is either longitudinal or transverse, rarely anything in between[15,17]. The explanation of component 2 as an oblique shock is therefore a specially contrived geometrical solution, which could occur once, but not regularly.

Table 1 Intensities of the three components in the bright head of 3C273 corrected for Doppler beaming

Component	Distance from core (arc s)	Blue-shift	Observed flux density (mJy)	Intrinsic flux density (mJy)
1	22.1	1.2	450	216
2	21.4	3.4	1,500	13
3	20.4	4.0	450	3

We believe that other explanations that could be put forward are less probable than the following suggestion.

(4) The aspect angle may be modified by relativistic aberration. The angle θ_s, calculated above from the polarization, is that measured in the co-moving frame of the source. If component 2 is moving relativistically, aberration changes an inclination angle from θ_{jet} to θ_{obs}, given by $\sin\theta_{jet} = \eta \sin\theta_{obs}$, where η, the Doppler blue-shift, is given by $\eta = \gamma^{-1}(1-\beta\cos\theta_{obs})^{-1}$. If we equate θ_{obs} with $\theta_0 = 12°$ deduced from the superluminal motion and θ_{jet} with $\theta_s = 47°$ deduced from the polarization, we obtain as the Doppler shift of component 2, $\eta \simeq 3.4$. The Doppler shift measured in the frame co-moving with the quasar is larger by a factor $(1+z)_{QSO}$, that is, $\eta = 3.96$. Relative to the quasar the speed of component 2 is $\beta \simeq 0.91$ and $\gamma \simeq 2.4$. If component 2 is partially compressed (a thick sheet) then $\theta_s > 47°$ and the values of β, γ and η given above should be regarded as lower limits.

We may apply these arguments to the precursor, component 1, which we suggest corresponds to the 'working surface' of the beam[10] and moves with the speed of the bow shock. From ram pressure arguments[2] this speed must be sub-relativistic, in the range $0.02 < \beta < 0.3$. It follows that most of the deceleration occurs between component 2 and component 1. If the velocity of component 1 is $\beta = 0.2$, the Doppler shift $\eta = 1.22$, and the inclination angle in the emitting frame $\theta_{jet} \simeq 14°$. The polarization predicted for a Laing sheet would then be 2%, equal to that observed in component 1. Simulation models[18] suggest that the working surface may be curved, in which case one may expect some polarization near the edge, but little in the centre of component 1. This description is entirely consistent with Fig. 1.

In this model, the relative intensities of the different components are altered by the Doppler beaming factor[8] of $\eta^{3+\alpha}$. The Doppler shift of component 3 is not known, but the maximum value consistent with $\theta_0 = 12°$ is 4.7, and if we assume a monotonic deceleration down the jet, η_3 must lie between 4.7 and $\eta_2 = 3.4$. We take $\eta_3 = 4$.

Applying a correction to the co-moving frame using Doppler shifts as above yields the intrinsic intensities given in Table 1, where it can be seen that the precursor is no longer a minor feature in front, but is intrinsically much the brightest component, as may be expected at the site of the greatest deceleration. Components 2 and 3 correspond to secondary features, which, if they were not amplified by beaming, would probably be classed as 'knots' similar to those found in many jets.

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1. Schmidt, M. *Nature* **197**, 1040 (1963).
2. Conway, R. G. *et al. Nature* **294**, 540-542 (1981).
3. Perley, R. A. in *VLBI and Compact Radio Sources* (eds Fanti, R., Kellerman, K. & Setti, G.) 153-156 (Reidel, Dordrecht, 1984).
4. Schmidt, G. D., Peterson, B. M. & Beaver, E. A. *Astrophys. J. Lett.* **220**, L31-L37 (1978).
5. Lelievre, G. *et al. Astr. Astrophys.* **138**, 49-56 (1984).
6. Willingale, R. *Mon. Not. R. astr. Soc.* **194**, 359-364 (1981).
7. Pearson, T. J. *et al. Nature* **290**, 365-368 (1981).
8. Blandford, R. D. & Konigl, A. *Astrophys. J.* **232**, 34-48 (1979).
9. Bridle, A. H. & Perley, R. A. *A. Rev. Astr. Astrophys.* **22**, 319-358 (1984).
10. Begelman, M. C., Blandford, R. D. & Rees, M. J. *Rev. mod. Phys.* **56**, 255-351 (1984).
11. Davies, J. G., Anderson, B. & Morison, I. *Nature* **288**, 64-66 (1980).
12. Cornwell, T. J. & Wilkinson, P. N. *Mon. Not. R. astr. Soc.* **196**, 1067-1086 (1981).
13. Conway, R. G. & Kronberg, P. P. *Mon. Not. R. astr. Soc.* **142**, 11-32 (1969).
14. Conway, R. G. & Stannard, D. *Nature* **255**, 310-312 (1975).
15. Laing, R. A. *Mon. Not. astr. Soc.* **193**, 439-449 (1980).
16. Davis, R. J., Stannard, D. & Conway, R. G. *Mon. Not. R. astr. Soc.* **185**, 435-440 (1978).
17. Laing, R. A. *Mon. Not. astr. Soc.* **195**, 261-324 (1981).
18. Wilson, M. J. & Scheuer, P. A. G. *Mon. Not. R. astr. Soc.* **205**, 449-463 (1983).

Computer classification of sea beds by sonar

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Direct sampling of the sea bed is not always feasible and can be unreliable because the samples obtained may not be representative. An alternative approach is to classify the superficial sea bed using the backscattered signal from side-scan sonars. The usual paper display contains less information than the signal creating it, because of its low dynamic range, and interpretation of sediment types is often difficult. There are also problems with statistical analysis of the signals[1]. The usual methods of pattern recognition cannot resolve small-scale features in otherwise homogeneous regions[2] and, although the backscattering strength is a useful quantity, meaningful numerical values can be obtained only with a calibrated system[3]. To overcome these shortcomings we have now developed a spectral analysis method that is insensitive to system gain calibration. The method retrieves information from the signals that is well below the usual power spectrum point, thus effectively widening the system bandwidth, and can discriminate between the signals returned from six sea-bed types.

It can be shown theoretically that the scattered sound field depends on both roughness and hardness of the sediment/water interface[4], but analytical expressions are unsuitable for most practical applications, not so much because of their complexity but because of the difficulty in quantifying roughness (statistically random corrugations) and hardness (mixed boundary conditions) of real sea beds. Although properties of scattered sound fields corresponding to an ensemble of sea-bed patches manifest only indirectly the combined effect of roughness and hardness on the incident sound field, they are nevertheless more suitable for our present semi-empirical approach. The shape of the probability distribution (of the scattered field intensity) does relate to the sea-bed roughness[5], but is not so sensitive to its hardness.

We now consider a side-scan sonar transmitting a rectangular pulse of length T ($\gg T_0$) at carrier frequency $\omega_0 (= 2\pi/T_0)$. Received signal amplitude is proportional to the pressure of the sound field at the transducer. The received signal is a quasi-harmonic stochastic function of time ('reverberation') given by $a(t)\cos[\omega_0 t + \psi(t)]$, where $a(t)$ and $\psi(t)$ are the amplitude and the phase of the signal respectively, which are both assumed to be slowly varying functions of time in comparison with $\cos\omega_0 t$. Our method uses $a(t)$ only and yields numerical features that are invariant under the linear transformation of $a(t)$ and hence independent of the signal d.c. level and gain.

We denote by $a_i(t)$ the signal amplitude within the ith rectangular window ($i = 1, 2, \ldots, n$) of a fixed time duration T_W ($\gg T$). The minimum value of the signal amplitude is set equal to zero so that $a_i(t) \geq 0$. The windows should have no temporal overlaps and can be taken from any number of pings. We further denote by $w(t)$ the time window function (other than rectangular) such that $w(t) = 0$ for $t < T_D$ and $t > T_D + T_W$, where T_D is the initial delay (measured from the instant of transmission). The resulting windowed signal can be written as $g_i(t) = w(t)a_i(t)$.

If the signal is digitized so that N samples are taken in each window with sampling interval Δt, we have $T_W = N\Delta t$. The Nyquist frequency is equal to $\pi/\Delta t$; if ω_B is the highest angular frequency of interest, the Nyquist sampling theorem implies $\Delta t \leq \pi/\omega_B$.

Performing the Fourier transform (operator $\mathcal{F}$) on the windowed amplitude $g_i(t)$ and taking the modulus squared of the resulting complex quefrency amplitudes, the ith power spectrum is obtained as $P_i(\omega) = |\mathcal{F}\{g_i(t)\}|^2$. By averaging n power values at each frequency, we obtain the averaged power spectrum $\langle P(\omega)\rangle = n^{-1}\sum_{i=1}^{n} P_i(\omega)$, where it is assumed that the system

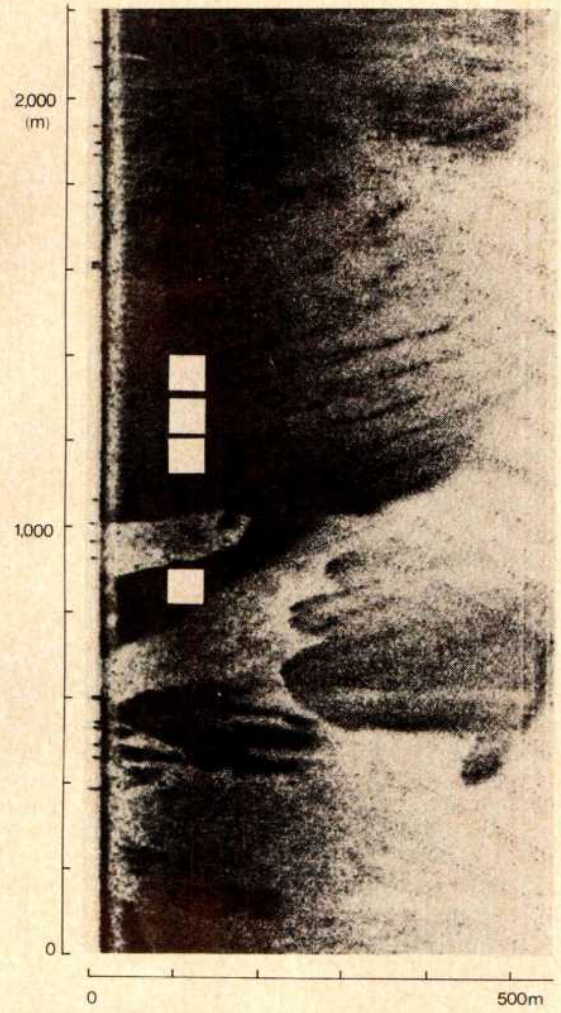


Fig. 1 Side-scan sonar paper record of sand and gravel sea-bed region. Blank squares indicate the approximate locations of four sample areas. The record width corresponds to 550 m range, the zero range being on the left-hand side. Sand and gravel appear as light and dark patches, respectively.

gain remains unaltered during the time taken to receive all n windows.

Performing the Fourier transform on the logarithm of the averaged power spectrum and taking the modulus squared of the resulting complex quantities, the power cepstrum (or simply 'cepstrum') is obtained $C(\tau) = |\mathcal{F}\{\log\langle P(\omega)\rangle\}|^2$, where the time-lag variable τ is called quefrency. The cepstrum, applied to various seismic, speech analysis and machine vibration problems, appears to be more useful in certain cases[6,7] than either the power spectrum or the auto-correlation function. However, we believe it has never been applied to side-scan sonar signals of 'reverberation' type not separating into 'echo' and 'noise'.

We define the power cepstrum integral (cumulative power cepstrum) by $I(\tau') = \int_0^{\tau'} C(\tau)\,\mathrm{d}\tau / \int_0^{T_W} C(\tau)\,\mathrm{d}\tau$, with the variable τ' in the range $\tau_0 \leqslant \tau' \leqslant T_W$, where τ_0 ($\leqslant T$) is a fixed interval.

We introduce two further parameters to characterize the power cepstrum integral, namely, the intercept and the slope at τ_0 given by $D_1 = 1 - I(\tau_0)$ and $D_2 = (\mathrm{d}/\mathrm{d}\tau')I(\tau')|_{\tau'=\tau_0}$, respectively, the cepstrum integral features, which both tend to zero when the cepstrum $C(\tau)$ contracts to the initial quefrency range $0 \leqslant \tau \leqslant \tau_0$.

The side-scan sonar, used for data acquisition, is a standard medium range system operating at 48-kHz carrier frequency, 1-ms pulse length, 2-kHz nominal receiver bandwidth and the usual time-varied gain. The amplitude envelope is recorded on magnetic tape in analog form with 48-dB dynamic range. Hardware for data processing consists of an 8-bit A/D converter and a standard 32 K microcomputer with a dual disk drive. The amplitude envelope is digitized at a sufficiently high rate and

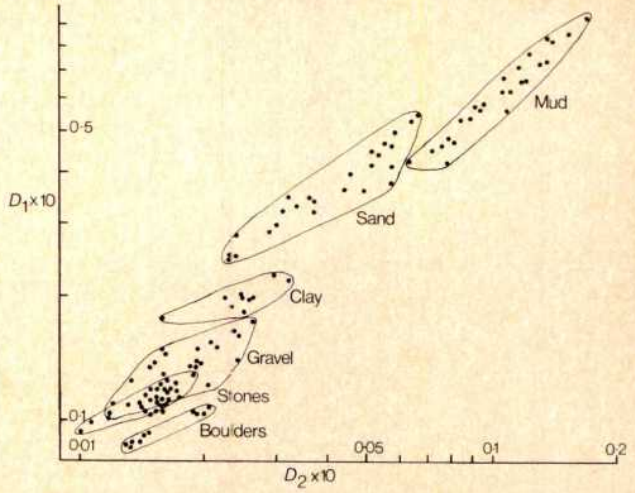


Fig. 2 Cepstrum integral features plane representation of all 120 sample areas. The boundaries to each sea-bed type are drawn by hand for clarity of display only. The time window $w(t)$ is chosen to be of the Hanning (cosine-squared) type. All Fourier transforms are performed according to the fast Fourier transform algorithm. The cepstrum is integrated numerically with a step equal to τ_0 and a straight line is interpolated through the first four points by the least-squares method in evaluating D_1 and D_2. The gain independence of D_1 and D_2 as evaluated by our software has been tested numerically; change in gain by a factor of up to four causes deviations in D_1 and/or D_2 that are at most 5%. The numerical values of D_1 and D_2 for a given sea-bed type depend on the number of windows n, especially when n is small; both sequences (for D_1 and D_2) that correspond to $n = 1, 2, 4, 8, 16, 32$ converge relatively fast so that little can be gained by going to the values of $n > 32$.

integer values in the range 0–255 saved on disk for further processing. A typical side-scan sonar paper record is presented in Fig. 1.

All of our 120 sample areas are homogeneous as far as could be ascertained in real sea beds, are from regions familiar from previous surveys and are free from noise and interference. Based on records (like that in Fig. 1), as well as other knowledge of the regions, any one of the sample areas is assigned to only one of the six classes: mud, sand, clay, gravel, stones and boulders. The sample areas are geographically distributed as follows: mud (25 from the South Baltic Sea), sand (5 from Land's End, 10 from the Outer Hebrides and 10 from the western English Channel), clay (10 from the southern North Sea), gravel (10 from southern North Sea and 15 from western English Channel), stones (10 from South Baltic Sea and 15 from the Outer Hebrides) and boulders (10 from South Baltic Sea).

Table 1 contains the arithmetical mean and standard deviation of the two cepstrum integral features (D_1 and D_2 multiplied by 10) for the six sea-bed types. Numerical values for all 120 sample areas are presented graphically (log–log scale) in Fig 2; each sample area corresponds to a single point in the two-dimensional plot of D_1 against D_2. Each sea-bed type corresponds to an area in (D_1, D_2) plane and the only overlap occurs between gravel and stones areas. Lower values of D_1 and D_2 generally

Table 1 Arithmetical mean and standard deviation of the cepstrum integral features for six sea-bed types

Sea-bed type	No. of sample areas	$D_1 \times 10$ Mean	$D_1 \times 10$ Dev.	$D_2 \times 10$ Mean	$D_2 \times 10$ Dev.
Mud	25	0.619	0.142	0.107	0.027
Sand	25	0.368	0.085	0.042	0.014
Clay	10	0.197	0.014	0.025	0.004
Gravel	25	0.135	0.018	0.018	0.004
Stones	25	0.111	0.007	0.015	0.002
Boulders	10	0.097	0.009	0.017	0.003

correspond to 'harder' sea-beds and the six sea-bed types span a factor of ~10 in the values of D_1 and D_2.

Judging by our results, it seems certain that the cepstrum and its integral features must have an important role in the classification of sea beds. The dependence of the discriminatory ability of the method on pulse length and carrier frequency is presently being investigated especially with respect to the classification of non-homogeneous sea-bed regions (for example, sand with gravel).

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1. Ol'shevskii, V. V. *Characteristics of Sea Reverberation* (Consultants Bureau, New York, 1967).
2. Pace, N. G. & Dyer, C. M. *IEEE Trans.* GE-17, 52-56 (1979).
3. Wong, H.-K. & Chesterman, W. D. *J. acoust. Soc. Am.* 44, 1713-1718 (1968).
4. Brekhovskikh, L. & Lysanov, Yu. *Fundamentals of Ocean Acoustics* (Springer, Berlin, 1982).
5. Stanton, T. K. *J. acoust. Soc. Am.* 75, 809-818 (1984).
6. Bogert, B. P., Healey, M. J. R. & Tukey, J. W. in *Proc. Symp. Time Series Analysis* (ed. Rosenblatt, M.) 209-243 (Wiley, New York, 1963).
7. Noll, A. M. *J. acoust. Soc. Am.* 41, 293-309 (1967).

Polarity reversal in the Solomon Islands arc

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McKenzie[1] proposed that the direction of subduction beneath an island arc will reverse following arc–continent collision. Dewey and Bird[2] suggested that such an arc polarity reversal occurred in northern New Guinea; Johnson and Molnar[3], however, proposed two alternative models of plate convergence that could explain the observed seismicity. We present here a spatial seismicity study of the Solomon Islands region which has revealed the existence of two juxtaposed Wadati–Benioff (W–B) zones of opposite polarity. Shallow and intermediate foci define a north-east-dipping W–B zone associated with active subduction along the New Britain and San Cristobal trenches and a south-west-dipping W–B zone associated with the inactive North Solomon trench (NST). These data provide the first direct seismic evidence of a reversal in subduction polarity at an island arc.

The Solomon Islands arc forms a linear double chain of islands north-east of the New Britain and San Cristobal trenches. The arc-trench system is part of the convergent boundary between the westward-moving Pacific Plate and the northward-moving Indo-Australian Plate (Fig. 1). Arc volcanics provide evidence of convergence between the Pacific and Indo-Australian plates in this region since the early Eocene. The polarity of the subduction is thought to have varied and a number of different models of the tectonic evolution have been proposed[4–7]. Bathymetric and seismic reflection data indicate a relict subduction zone dipping south from the North Solomon and Vitiaz trenches[8,9]. The abnormally thick lithosphere of the Ontong Java Plateau collided with the NST sometime during the Miocene, although the exact timing remains controversial[4,7,9]. Following the collision, the arc polarity reversed and the present regime of north-east subduction at the New Britain, San Cristobal and Vanuatu trenches began by 10 Myr BP. The hiatus in island arc volcanism lasting from late-early to early-late Miocene observed throughout the Solomon Islands[7,10] may be evidence of a temporary cessation of subduction during the polarity reversal.

Earthquake hypocentres, volcano locations and major tectonic features of the Solomon Islands region are illustrated in Fig. 2. Hypocentres covering the period 1 January 1964 to 30 June 1984 are taken from the International Seismological Centre (ISC) catalogue and include all events with body-wave magnitude ≥4.7 and recorded by more than 15 stations. Three primary features are evident from Fig. 2. First, the spatial density of earthquake foci changes along strike; shallow (<70 km) and intermediate (70–300 km) activity is concentrated in two zones of greatest density under the northwestern and southeastern parts of the arc. Second, a zone of low-density shallow seismicity is associated with the active spreading system extending east across the Woodlark Basin. The low density of seismicity in the central Solomon Islands (from 156–159 °E) coincides with the site of subduction of the young Woodlark Basin lithosphere. Third, diffuse shallow and intermediate seismicity is also observed north-east of New Georgia and Guadalcanal, extending to Santa Isabel and Malaita, which provides evidence of lithosphere previously subducted at the NST.

Four representative seismicity cross-sections located in Fig. 2 and one longitudinal section are shown in Fig. 3. The top of the subducted lithosphere is assumed to extend from the topographic trench axis downward along the upper limit of the concentrated seismicity. Both the dip and the depth-extent of the north-east-dipping W–B zone associated with subduction at the New Britain and San Cristobal trenches vary significantly along strike (Figs 2, 3). North of Bougainville, Fig. 3A shows a well-defined seismic zone dipping at an initially shallow angle, then steepening rapidly until becoming almost vertical below 100 km. Teleseismic activity is virtually absent in the overriding

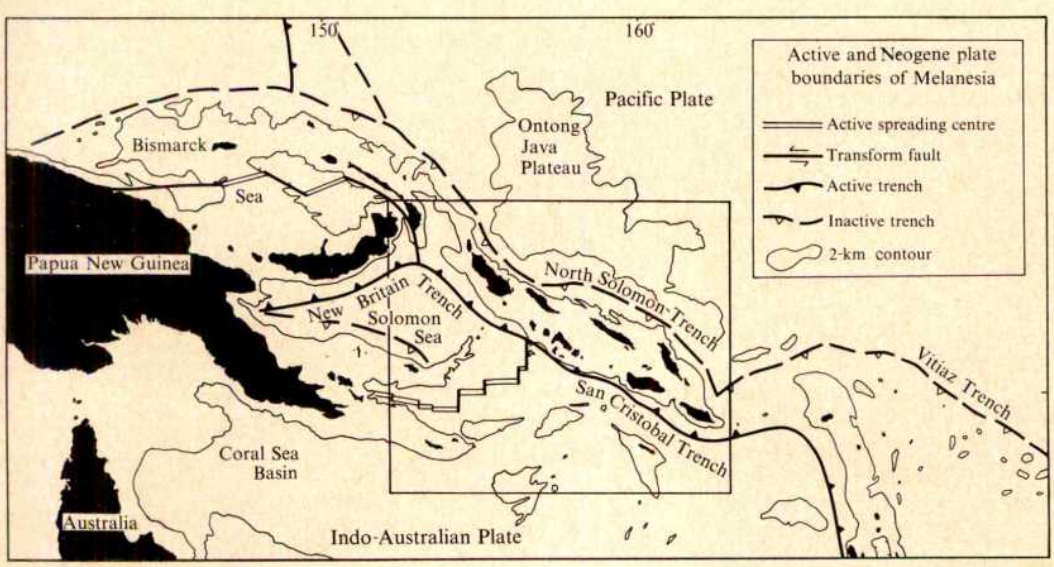


Fig. 1 Summary of active and Neogene plate boundaries of Melanesia (after ref. 19). Box shows location of Fig. 2.

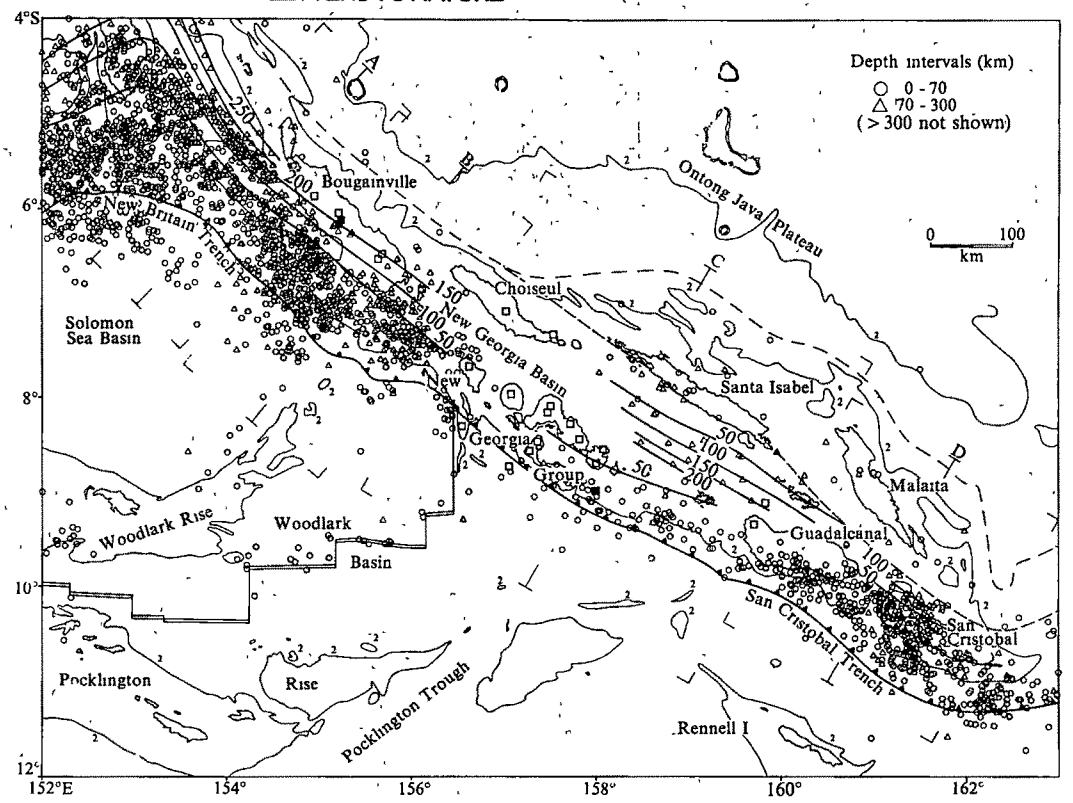


Fig. 2 Regional seismicity (ISC > 4.7 M_b, 1 January 1964 to 30 June 1984) and Wadati–Benioff zone contours (at 50-km intervals) for the Solomon Islands. The Woodlark spreading system is shown following Taylor[13] by double lines (spreading segments) and single lines (transforms). The dashed-dotted line marks the North Solomon trench; the dashed line indicates the Kaipito–Korighole fault. Large squares locate Plio-Pleistocene volcanoes; filled squares are active volcanoes.

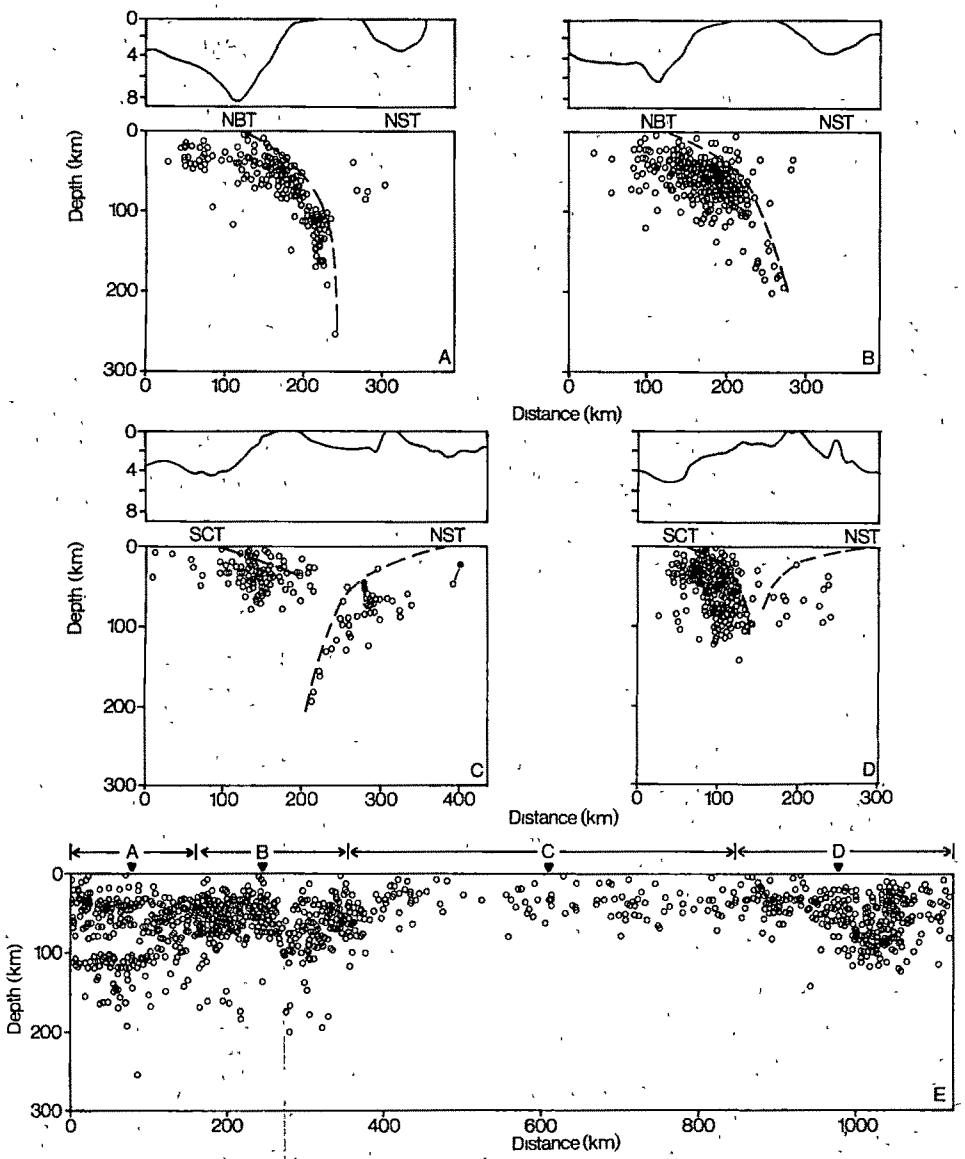


Fig. 3 Projections on vertical planes (located in Fig. 2) of ISC seismicity. The solid circle in C (top right, ○—●) represents a relocated earthquake. Dashed lines locate the inferred top of subducted lithosphere. Profile E is subparallel to the strike of the Solomon Islands, extends from 5.5° S, 153.5° E to 11.0° S, 162.0° E with a width of 425 km, and excludes the hypocentres associated with the south-west-dipping W–B zone. The New Britain, San Cristobal and North Solomon trenches are indicated by their initials.

plate. Near Bougainville (Fig. 3B) the dip of the seismic zone is less steep and the zone has become more diffuse. The W-B zone in these two profiles is continuous to just over 200 km, below which there is a seismic gap. The active volcanoes on Bougainville occur about 150 km above this W-B zone. Deep earthquakes occur throughout the Solomon Islands in cigar-shaped zones at depths of 400 ± 20, 500 ± 20 and 535 ± 10 km, although there is little indication that the presently active W-B zone includes the deep seismicity.

Profile C (Fig. 3C) traverses the New Georgia Group. Subduction of the young lithosphere and active spreading system of the Woodlark Basin is characterized by shallow diffuse low-magnitude seismicity. Similar seismicity characteristics are observed in the region where the Chile Rise is being subducted beneath South America[11]. There, the spreading segments trend parallel to the trench axis and, adjacent to the triple junction, there is a 3° gap in the chain of active volcanoes[12]. In contrast, in the Solomon Islands the spreading segments trend sub-parallel to the convergence direction and, adjacent to the triple junction, there is a maximum in the volcanism of the island arc[13]. Although the seismicity east of the Woodlark Basin does not extend below 80 km, voluminous eruptions of Plio-Pleistocene lavas have formed the New Georgia Group and the active submarine volcano, Kavachi, within 30-90 km of the trench. Fig. 3D shows a north-east-dipping almost vertical seismic zone, continuous to just over 100 km, beneath San Cristobal. A relict south-west-dipping slab, discussed below, may force the presently subducting lithosphere into its almost vertical attitude.

A number of events in Fig. 3C, D define a south-west-dipping W-B zone that we attribute to the relict slab of Pacific Plate lithosphere formerly subducted at the NST. Several hypocentres in the shallow part of this zone were relocated by a joint hypocentral determination technique[14] to depths significantly shallower than those reported by ISC and we note a similar systematic mislocation of events beneath the Japan Trench[15]. We have therefore drawn the top of the W-B zone above the apparent upper level of concentrated seismicity. Figure 3C clearly shows a south-west-dipping W-B zone. Curtis[16], using data spanning 1961-70, noted that diffuse seismicity south of Santa Isabel seemed to form a south-west-dipping zone, but that it could conceivably be incorporated into a north-east-dipping W-B zone if the foci near New Georgia were included. Denham[17] suggested that the Pacific Plate is underthrusting Santa Isabel; there are no cross-arc seismicity zones, however, to indicate that it is connected to the active plate boundaries to the south. Either the seismicity is entirely relict[18], or the presently subducting lithosphere may be pushing the old slab deeper into the asthenosphere, forcing re-equilibration. It may be that the lack of intermediate earthquakes associated with the subduction of a spreading system beneath the central Solomon Islands affords us the unusual clarity in observing the relict slab.

The recognition that this relict slab is present has significant implications also for current petrological studies. Recent volcanism on Savo is located 100-150 km above the relict W-B zone and the Plio-Pleistocene volcanoes of Choiseul (Mt Matambe and Kamboro Peak) are in a similar tectonic position along the strike of this W-B zone, from Savo (Fig. 2). These volcanoes may be evidence of a reactivation of magmatism associated with the relict slab.

Received 30 July, accepted 21 December 1984

1 McKenzie, D. P *Geophys J* **18**, 1-32 (1969)
2 Dewey, J F & Bird, J M J *Geophys Res* **75**, 2625-2647 (1970)
3 Johnson, T & Molnar, P. J *Geophys Res* **77**, 5000-5032 (1972)
4 Coleman, P J & Packham, G H *Earth Sci. Rev* **12**, 197-233 (1976)
5 Weissel, J K, Taylor, B & Karner, G D *Tectonophysics* **87**, 253-277 (1982)
6 Falvey, D A. & Pritchard, T in *Trans Third Circum-Pacific Council Energy Mineral Resources* 593-599 (1984)
7 Kroenke, L W *Cenozoic Tectonic Development of the Southwest Pacific* (CCOP/SOPAC Technical Bull 6, in the press).
8 Brocher, T in *Earth Science Ser Circum-Pacific Council Energy Mineral Resources* (eds Brocher, T & Holmes, R) (in the press)
9 Kroenke, L. W thesis, Univ Hawaii (1972).
10 Coleman, P J. *Pacif Sci.* **24**, 289-314 (1970)
11 Forsyth, D. W. *J Geophys Res* **80**, 1429-1914 (1975)
12 Herron, E M, Cande, S C & Hall, B R in *Nazca Plate Crustal Formation and Andean Convergence* (eds Kulm, L D, Dymond, J, Dasch, E J. & Hussong, D M) 683-701 (Geol Soc Am, Mem 154, 1981)
13 Taylor, B in *Earth Science Ser Circum-Pacific Council Energy Mineral Resources* (eds Taylor, B & Exon, N) (in the press)
14 Dewey, J. W thesis, Univ California, Berkeley (1971)
15 Hirata, N, Yamada, T, Shimamura, H, Inatani, H & Suyehiro, K *Geophys J R. astr Soc* **73**, 653-669 (1983)
16 Curtis, J. W *J geol Soc. Aust* **20**, 1-19 (1973)
17 Denham, D *Bull Aust Explor Geophys* **6**, 78-79 (1975)
18 Toksoz, N *Sci Am.* **233**, 88-98 (1975)
19 Taylor, B & Karner, G D *Rev Geophys Space Phys* **21**, 1727-1741 (1983)

A 'northern oscillation' relating northern hemispheric pressure anomalies and the Indian summer monsoon?

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Analysis of the causes of the Indian monsoon have concentrated on climatological phenomena in the southern hemisphere, such as El Niño and the Southern Oscillation. By contrast, we have examined meteorological records for the northern hemisphere (0°–180°) spanning much of this century. We find that zonally-integrated mean sea-level pressure anomalies across Eurasia during January to April exhibit a statistically significant negative correlation between values for higher and sub-tropical latitudes. We further find that there is an association between above-normal activity of the Indian monsoon and a steep poleward-directed pressure anomaly gradient, which tends to persist through the summer and may indicate a strong zonal flow in the circumpolar westerlies. On the other hand, when this pressure anomaly gradient reverses, zonality is disturbed and the Indian monsoon tends to be drier than usual. Under these conditions, the circumpolar flow becomes persistently meridional as a result of a predisposition to blocking and splitting of the jet.

Exner[1] identified an inverse relationship between mean sea-level pressure in winter (December, January and February) over the northern hemispheric Eurasian Arctic and that in the central Mediterranean. Although he visualized a possible relationship with tropical precipitation, Exner did not pursue further analysis owing to the paucity of data. Through extensive correlation analysis, Walker[2,3] related anomalies in the global centres of action, especially Icelandic and Aleutian, a few months (even a season) ahead with subsequent performance of the summer monsoon over India. Although Walker recognized the importance of Exner's analysis, he emphasized the pressure anomalies

Table 1 Correlation matrix of mean sea-level pressure anomaly zonally integrated across Eurasia (0–180°)

Lat. °N	80°	70°	60°	50°	40°	30°	25°
80°	1 00	0.77	0.32	−0.15	−0.27*	−0 22*	−0 20*
	(1.00)	(0.76)	(0 27)	(−0 11)	(−0 10)	(0 01)	(0 02)
70°		1.00	0 75	0.11	−0.32*	−0 40*	−0 38*
		(1 00)	(0 71)	(0.06)	(−0.26)†	(−0 31)†	(−0 30)†
60°			1 00	0 59	−0.09	−0 39*	−0.38*
			(1 00)	(0.59)	(−0 03)	(−0 40)*	(−0.41)*
50°				1.00	0.57	0.05	−0.08
				(1.00)	(0.59)	(0 02)	(−0.11)
40°					1 00	0 70	0.48
					(1 00)	(0 64)	(0.45)
30°						1 00	0.91
						(1.00)	(0.92)
25°							1 00
							(1.00)

Upper line: January–April; 340 data points. Lower line (in parentheses): January + February + March + April, 85 data points
* Significant at 0 1% level
† Significant at 1% level

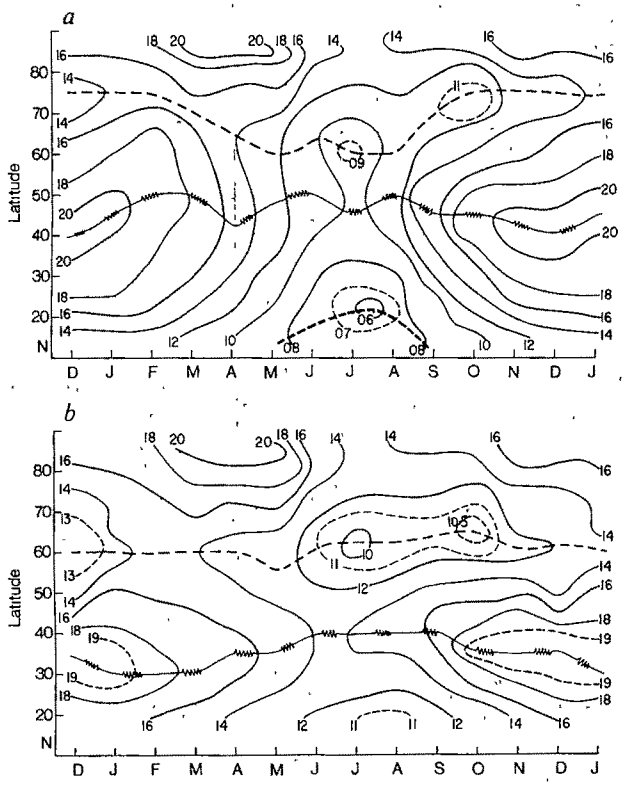


Fig. 1 Annual variation of the profile of mean sea-level pressure (mbar, first two digits omitted) over the northern hemisphere, zonally integrated: *a*, across Eurasia (0–180°; predominantly continental); *b*, around the northern hemisphere (oceanic and continental). Average for 85 yr (1900–84). ——·——·——, Axis of sub-Arctic trough; ——∿——∿——, axis of sub-tropical ridge; --------, axis of tropical trough.

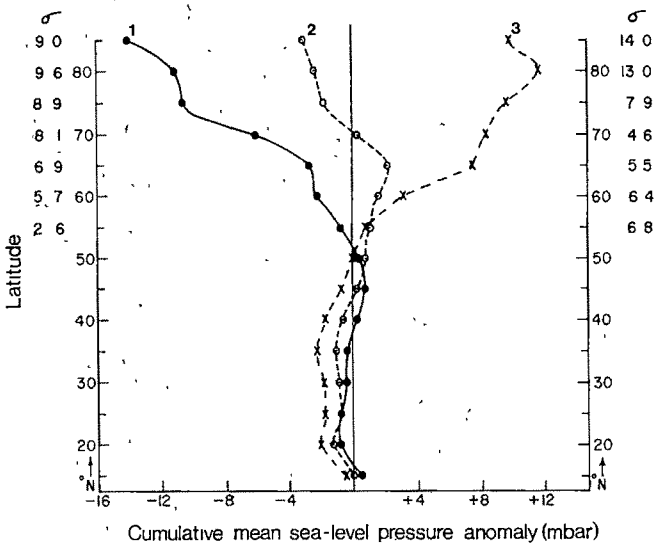


Fig. 2 Latitudinal profile of cumulative anomaly of mean sea-level pressure (January–April), zonally averaged across Eurasia (0–180°) and composited for three categories of performance of the Indian summer monsoon (1) above normal (≥+6%), 12 yr; (2) normal (≥−5%–≤+5%), 18 yr; (3) below normal (≤−6%), 12 yr. Standard deviation (σ) of the two contrasting samples is given with respect to the latitude, on either side. The two samples are significantly different. Note the following: (i) rainfall departures are available for north-west India and the Peninsula for the period 1940–81. (ii) In the almost 100-yr data set supplied by the UK Meteorological Service, data gaps have been reported over USSR between 1916 and 1921 and 1938–39. Hence, the data-gap-free period of 42 yr (1940–81) was chosen for preparation of the composites. (iii) 5% is half of the standard deviation of long-term rainfall of India.

in the centres of action of the southern hemisphere—the Southern Oscillation—and their teleconnections with Indian monsoon rainfall. Thereafter, particularly after Bjerknes[4], more emphasis has been laid on the relationship between the Southern Oscillation/El Niño and the activity of the Indian summer monsoon. Ramage[5] has drawn attention to the limitations of correlation analysis, in which a centre of action undergoes a significant change of location, resulting in a change of the sign of correlation. Therefore, we re-examine here the northern hemispheric pressure anomalies, especially those of continental Eurasia, with an analysis procedure that not only offsets distortions arising from positional changes in the centres of action but also filters sub-synoptic scale noise.

Mean sea-level pressure data for 26 yr (1951–76) received from the Chinese Academy of Sciences (Tao Shi Yan, personal communication), followed subsequently by more extensive data for 85 yr (1900–84) from the UK Meteorological Service formed the principal data sources for the study. Profiles of the monthly march of mean sea-level pressure, zonally integrated across Eurasia (0–180°; predominantly continental) and around the northern hemisphere (0–360°; oceanic and continental) in 5°-lat. steps from 85° N to 15° N are presented in Fig. 1. In the Eurasian profile (Fig. 1*a*), the sub-Arctic trough moves towards the Equator from 75° N in January to 60° N in July and has a higher value of mean pressure in winter compared with that in summer and autumn. The sub-tropical ridge located around 50° N in February/March shifts to 45° N in July and thence to 40° N in December. Again, the mean pressure values are higher in winter than in summer. In the average profile, the sub-tropical high is substantially effaced and is seen as a weak ridge in summer at sea level. The axis of the sub-Arctic trough approaches the position of the weak sub-tropical high belt during May–August. The tropical trough is sharply delineated, traversing from 15° N in May to near 25° N in July/August and shifting equatorward thereafter. In contrast, the axes of the sub-Arctic trough and the sub-tropical ridge in the hemispherical profile (Fig. 1*b*) are about 5–15 degrees of latitude equatorward. The tropical trough is relatively less well defined.

Space-time sections of Eurasian and hemispherical averages of mean sea-level pressure anomalies (January 1900–April 1984) gave the first indication of periodic reversals of pressure anomalies, particularly in winter and early spring, over the Arctic, poleward of 55° N. To express this quantitatively, a correlation matrix (Table 1) compiled with pressure anomaly values from January to April, serially and cumulatively is presented. A marked inverse relationship is noticed between pressure anomalies over higher/middle latitudes and those of the sub-tropics/tropics. The relationship is statistically significant at the 0.1% level. The negative association is striking between 70°/60° N and 30°/25° N. The matrix further suggests that a similar inverse relationship exists between pressure anomalies of the tropics of the northern and the southern hemispheres respectively.

To look for an association with the performance of the summer monsoon over India, the 42-yr data-gap-free period 1940–81 was chosen from the almost 100-yr sample. Monsoon seasonal rainfall departures (June–September) from the long-term norm for the plains of India, north-west India and the Peninsula are used to group the 42-yr period into three categories of monsoon performance over India, namely, 12 yr each of above normal (≥+6%) and below normal (≤−6%) and 18 normal years (≥−5% to ≤+5%). (Note that 5% represents half the standard deviation of long-term monsoon rainfall.)

The composited cumulative pressure anomaly profiles (January–April) display contrasting characteristics (Fig. 2). During this season, preceding above-normal monsoon performance, a distinct negative anomaly exists, with a steep gradient of ~15 mbar directed poleward from the domain of the sub-tropical

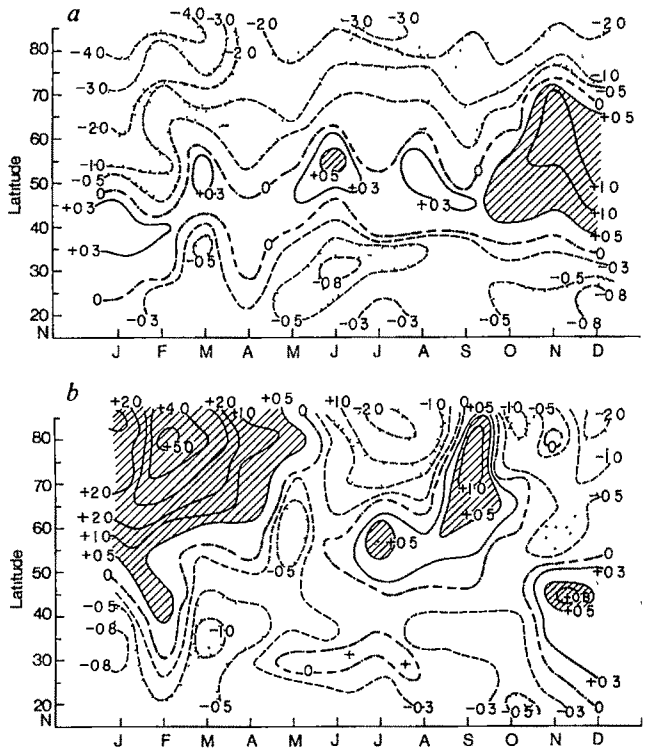


Fig. 3 Monthly march of mean sea-level pressure anomaly, zonally averaged across Eurasia (0–180°) and composited for: *a*, above normal monsoon (≥ +6%, 12 yr); *b*, below normal monsoon (≤ −6%, 12 yr). Note the following: (i) in *a* isopleths of negative and positive anomaly run zonally; in *b* isopleths of pressure anomaly display a meridional orientation. (ii) Positive rainfall anomaly during above-normal (good) monsoon years is ~+6–+8%, the largest being +18% in 1961. However, during below-normal (aberrant) monsoon years, rainfall departure is appreciably negative, ranging from −12% to −33%. (iii) Positive mean sea-level pressure anomaly over the Arctic poleward of 45° N is conspicuously large during January–April in highly aberrant monsoon years with rainfall anomaly < −16% of normal. (iv) Note the contrasting pressure anomaly distribution in summer, equatorward of 40° N, in relation to the opposing pressure anomaly over middle and higher latitudes during January–April.

ridge (45° N). On the other hand, when the summer monsoon performance is below normal, the antecedent anomaly pattern reverses and is diametrically opposite; the gradient of pressure anomaly of ~13 mbar, is now directed equatorward from the Arctic to 45° N. The curve antecedent to normal monsoon behaviour lies in-between the two contrasting profiles. The standard deviation (σ) of the two 12-yr samples, noted on either side of Fig. 2, shows a general decreasing tendency from the Arctic (85° N) to the middle latitude (55° N); the associated standard error is small. The mean $\pm\sigma/2$ values show that the two samples retain their significantly dissimilar characteristics. Application of 'Students' t test confirms that the two samples are statistically significant, poleward of latitude 60° N, with $P < 0.05$.

Using the entire series (1900–84), profiles were constructed with pressure anomalies averaged across Eurasia and around the northern hemisphere corresponding to 19 above-normal and 19 below-normal monsoon years. These two sets of profiles display characteristics essentially similar to those in Fig. 2 as well as statistical tests that are significant.

Figure 3 presents a composite of monthly march of pressure anomaly across Eurasia for extremes of monsoon performance. In the above-normal monsoon composite (Fig. 3*a*), a conspicuously large negative pressure anomaly is observed over the Arctic region poleward of 40° N in the preceding winter and early spring (January–April), persisting through the summer months and even thereafter. Immediately equatorward, the sub-tropical high is enhanced, but in the tropical trough region, equatorward of 40° N, during the ensuing summer (especially June–August) a negative anomaly, almost reaching ~ −1.0 mbar is observed. Considering that the composite is an average of 12 above-normal years and covers one half of the northern hemisphere, a negative pressure anomaly of this magnitude at low latitude is significant. The associated summer monsoon turns out to be active.

However, in the below-normal monsoon composite (Fig. 3*b*), the pronounced positive pressure anomaly of up to +5 mbar during January–April suggests a weakened Arctic trough. The sub-tropical ridge is totally annihilated and, in the ensuing summer, the tropical trough, equatorward of 40° N, is now characterized by a weak positive pressure anomaly. The associated summer monsoon activity is naturally suppressed.

Composites for contrasting monsoon years, prepared with anomalies of 500-mbar topography integrated across Eurasia (0–180°) and around the northern hemisphere, using data for the period 1951–79[6,7], also display characteristics similar to those in Fig. 3. A significantly large positive topographic anomaly overlies a positive mean sea-level pressure anomaly and vice versa.

Blocking, over high latitudes of Eurasia, was recently associated with monsoon droughts over India[8,9]. Arising from dynamical causes, the relatively more-frequent antecedent blocking, over west Eurasian high latitudes, was observed to extend from the lower stratosphere to sea level. Painting[10] reported increased blocking during winter in association with a positive mean sea-level pressure anomaly over high latitudes of the northern hemisphere. The positive pressure anomaly during January–April (Fig. 3*b*) could, therefore, be attributed to persistent high-latitude blocking over Eurasia. Marked differences were reported in hemispherical circulation characteristics over higher latitudes and the tropics during August, the typical summer month, in years of contrasting monsoon performance[8]. Thus, contrasting winter and early spring abnormality in pressure at mean sea level and in the overlying troposphere of the middle and higher latitudes of Eurasia seems to predetermine, to a great extent, the probable pressure anomaly distribution over low-latitude tropics of south Asia in the ensuing summer and the associated monsoon performance. A simple linear relationship does not, however, emerge between the magnitudes of the negative/positive pressure anomalies before the monsoon and subsequent monsoon rainfall departure. Work in progress to compile a suitable pressure oscillation index for the Eurasian region with the long-time pressure data series may provide further insights into this complex problem.

This study does not presume to preclude the apparent effects of El Nino or the Southern Oscillation on the behaviour of the summer monsoon over the Indian subcontinent. The role of soil moisture over high ground of south Asia and the contribution of sensible heat could be among the other factors leading to the organization of the monsoon trough (D. E. Jones and C. K. Folland, personal communication). This investigation just suggests that interaction may exist between antecedent pressure anomalies over middle and high latitudes of the northern hemisphere—the Northern Oscillation?—and the Indian summer monsoon. Much further work remains to be done.

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1 Exner, F M *Sitz.d.k.Akad. der Wissenschaften, Wien* **CXXII**, 1165–1240 (1913).
2 Walker, G. T *Mem India Met Dep* **24**(IV), 75–131 (1923).
3, Walker, G T. *Mem. India Met Dep* **24**(IX and X), 275–345 (1924)
4 Bjerknes, J *Mon Weath. Rev* **97**, 167–172 (1969)
5 Ramage, C. S *J Clim* **3**, 223–231 (1983).
6 *Die Grosswetterlagen Europas* (Deutscher Wetterdienst, Offenbach, 1953–79)
7 *Nordhemisphärische Wetterlarge* (Beilage zur Berliner Wetterkarte, 1960–79)
8 Raman, C R V & Rao, Y P. *Nature* **289**, 271–273 (1981)
9 Raman, C R V *Proc. Monex Symp Bali* (WMO) I, 21–25 (1982)
10 Painting, D J *Met. Off., Lond., Sci Pap* No 35, 1–25 (1977)

Stable isotope data and depositional environments in the late Quaternary Arctic Ocean

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There has been much speculation about the history of the Arctic Ocean, particularly its response to the late Quaternary climatic fluctuations[1–8]. As a result, considerable data have been gathered from Arctic Ocean sediment cores to reconstruct glacial and interglacial Arctic Ocean palaeoenvironments[8,9]. But even with these data the reconstructions and the correlations with the Quaternary chronostratigraphy have been unsatisfactory, mainly because of the lack of detailed stratigraphical data such as those provided by stable oxygen-isotope stratigraphy[10]. Here we present stable isotope records from Arctic Ocean sediment cores which can be correlated convincingly with corresponding data from the North Atlantic. Together with lithostratigraphical data, they provide new evidence on Arctic Ocean history in relation to global late Quaternary climatic fluctuations.

Our two sediment cores were collected during the Fram-I expedition[11]. Core Fram-I/4 was raised from the Fram Basin (84°29′56″ N, 08°58′39″ W, water depth 3,820 m) and core Fram-I/7 from the northern flank of Nansen Ridge (83°52′38″ N, 06°57′17″ W, water depth 2,990 m) (Fig. 1). The stable isotope measurements were made on planktonic foraminiferal shells of sinistrally coiled *Neogloboquadrina pachyderma*, with samples containing 55 individuals on average. Measurements followed standard procedures described elsewhere[12,13]. The O-isotope records in the upper parts of both cores (Fig. 2) offer clear evidence of the last glacial–interglacial transition, called Termination I (ref. 14), which is subdivided into Terminations I_A and I_B as defined by Duplessy *et al.*[15]. Additionally, the marked increase in $\delta^{18}O$ in core Fram-I/4 just below Termination I_B may correspond to the Younger Dryas[16]. The difference between the $\delta^{18}O$ shifts at the level of Terminations I_A and I_B in the two cores (Table 1) may be a result of different sampling intervals, that is, the larger sampling intervals of core Fram-I/7 may have resulted in a smoothing of the $\delta^{18}O$ record. On the other hand, bioturbation effects cannot be excluded. Nevertheless, the absolute shifts in $\delta^{18}O$ of ~2.7‰ for the whole of Termination I in both cores exceed the actual 'ice-effected' value of 1.6–1.8‰[17]. This ~1‰ excess may indicate a temperature increase of 4 °C. More probably, however, we may assume there was a direct supply of meltwaters derived from the Arctic ice during deglacial warming, which would indicate a decrease of salinity of ~0.6‰[18].

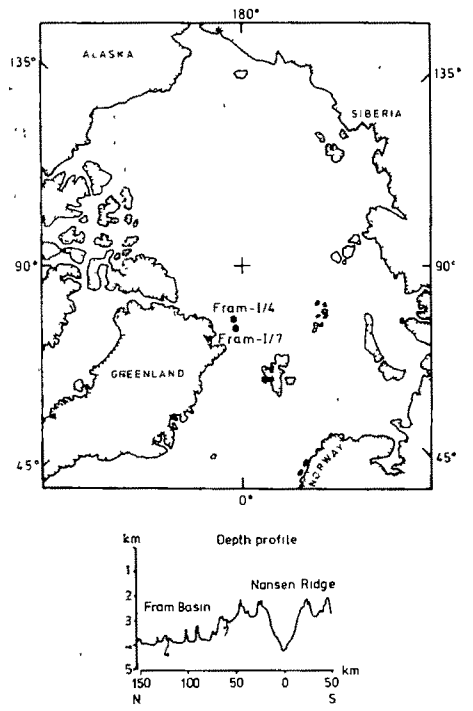


Fig. 1 Locations of cores Fram-I/4 and -I/7 The depth profile traces the bathymetry under the drift of ice station 'FRAM-I'

Furthermore, both cores exhibit a pronounced trend towards lighter $\delta^{18}O$ values in their lower parts, which may represent the O-isotope stage boundary 2/3. The strongly negative $\delta^{18}O$ peak during stage 2 in core Fram-I/7, however, is hard to explain in terms of global warming and its absence from the $\delta^{18}O$ record of core Fram-I/4 may indicate the presence of displaced sediments. Such sediments may have been brought in by turbidity currents, which are likely to occur on flanks of submarine ridges like the one from which core Fram-I/7 was raised (Fig. 1).

At present there are no ^{14}C data for either Fram core, but the O-isotope records resemble those of cores from low-latitude locations[10,19]. Therefore, because of this and the relatively short oceanic mixing time, it seems feasible to transfer the chronostratigraphical fixpoints from ^{14}C-dated low-latitude cores[15,20–22] to the O-isotope analogues of cores Fram-I/4 and Fram-I/7 (Fig. 2, Table 1). The average sedimentation rates between the top of Termination I_B and the stage boundary 2/3, which can be calculated from these fixpoints, are ~1.8 and ~2.6 cm × 10^{-3} yr for cores Fram-I/4 and Fram-I/7, respectively, and

Table 1 Chronostratigraphy, $\delta^{18}O$ shifts and sedimentation rates of cores Fram-I/4 and -I/7

Termination I		Fram-I/4			Fram-I/7		
	^{14}C age (kyr)	Core depth (cm)	$\delta^{18}O$ shift (‰)	Sedimentation rates (cm kyr^{-1})	Core depth (cm)	$\delta^{18}O$ shift (‰)	Sedimentation rates (cm kyr^{-1})
Termination I_B	8.5	3 0			13.0		
	10.0	5.0(?)	+0.75	1.33	17.0	+1.92	2.67
Younger Dryas			−0.56	2.25			
	10.8	6.8(?)					
Termination I_A	13 5	12.0	+1.33	1.60	23.0	+0 59	1 20
	16.0	16.0			26 0		
O-Isotope stage boundary							
1/2	13.5[20]	12 0	+2.67	1.74	23.0	+2.75	1.73
			(for entire Term I)			(for entire Term. I)	
2/3	27 0[21]	36.5(?)	−0.41	1.89	79 5(?)	−0.57	3.37*
				(for stage 2)			(for stage 2)

Data for Termination I are from refs 17, 22.
* Stage 2 sedimentation rates in core Fram-I/7 were estimated without a section between 54 and 65 cm, which is suggested to represent displaced sediments (see text).

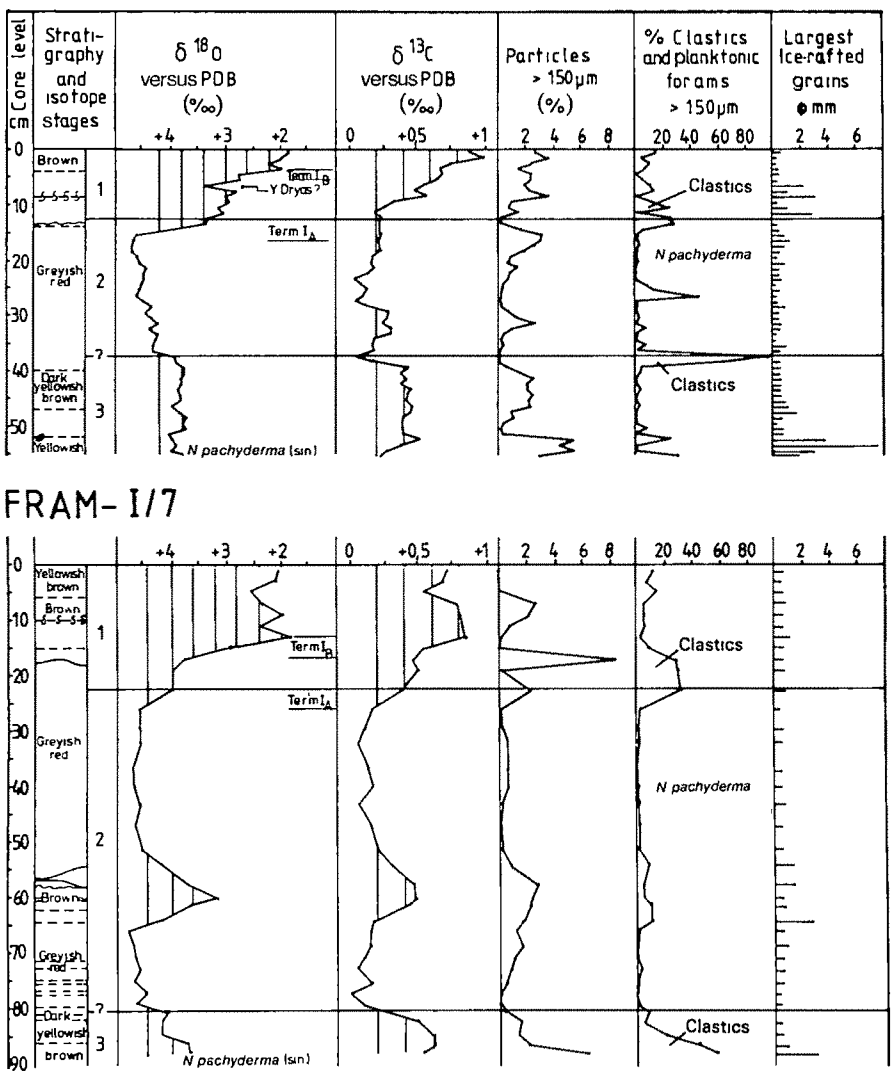


Fig. 2 Stable isotope records and lithostratigraphies of cores Fram-I/4 and -I/7. $\delta^{18}O$ values are shown relative to the PDB reference standard.

much higher than those previously reported for central Arctic Ocean sediments[23,24]. However, sedimentation rates of a similar order of magnitude have been reported recently[25].

Finally, the similarity in O-isotope data between our Arctic Ocean cores and those from the adjacent Norwegian-Greenland Sea[26,27] should be mentioned (Fig. 3). During stage 2 the O-isotope curves actually coincide without any significant difference, suggesting similar surface-water conditions in both deep-sea basins. During O-isotope stages 3 and 1, the Arctic cores tend towards lighter $\delta^{18}O$ values, perhaps because of a more pronounced meltwater effect in the Arctic Ocean than in the Norwegian-Greenland Sea.

The most prominent feature in the $\delta^{13}C$ records of both cores is the steady increase towards heavier values during Termination I (Fig. 2). This increase seems to start somewhat earlier in core Fram-I/7 than in core Fram-I/4, probably because of the different sampling intervals applied on the cores; stage 3 also exhibits heavier values than stage 2. The distinct increase in $\delta^{13}C$ in core Fram-I/7 during stage 2, as well as the $\delta^{18}O$ decrease in the same core section described above, does not occur in the $\delta^{13}C$ record of core Fram-I/4; this may support the assumption that this part of the section has been affected by sediment displacement.

Because $\delta^{13}C$ variations generally follow variations in hydrological parameters such as O_2 content[28], the increasing ^{13}C content in planktonic *N. pachyderma* (sin) shells may indicate the penetration of comparatively well-oxygenated surface-subsurface waters into this general region. This suggests that present-day circulation patterns in the Arctic Ocean may have begun during Termination I, a suggestion strongly supported by $\delta^{13}C$ values of *N. pachyderma* (sin) from Norwegian Sea cores[29] where a similar increase starts somewhat before Termination I. This may reflect the retreat of the glacial-stage ice cover with succeeding inflow of temperate North Atlantic Drift water into the Norwegian-Greenland Sea and, with respect to the $\delta^{13}C$ signal in our cores, into the Arctic Ocean through the Fram Strait[30]. Even the strong $\delta^{18}O$ shift in both cores during Termination I may in part be attributable to an increase in water temperature, indicating the influence of these warm waters.

The sediment compositions of the two cores correspond closely with their stratigraphy, permitting a correlation of their lithostratigraphies also. The lithologies may briefly be characterized as follows: the Upper Holocene sediments contain small amounts of $CaCO_3$ and coarse-grained ice-rafted material, whereas in the sediments, which were deposited during Termination I, that is, the global deglaciation, concentrations of these components are markedly increased. In the deposits corresponding to O-isotope stage 2, that is, fully glacial conditions, coarse ice-rafted material is less abundant, whereas concentrations of $CaCO_3$ are equal to or even higher than those in the Holocene sediments. Only in the section of core Fram-I/7, which has already been suggested to represent displaced sediments, are amounts of both components increasing. In the assumed O-isotope stage 3 sediments, the proportions of the coarse fractions and the amounts and grain sizes of ice-rafted material are clearly increasing, whereas concentrations of $CaCO_3$ are decreasing. The coarse fractions throughout both sediment cores consist mainly of planktonic foraminifers (mainly *N. pachyderma* (sin))

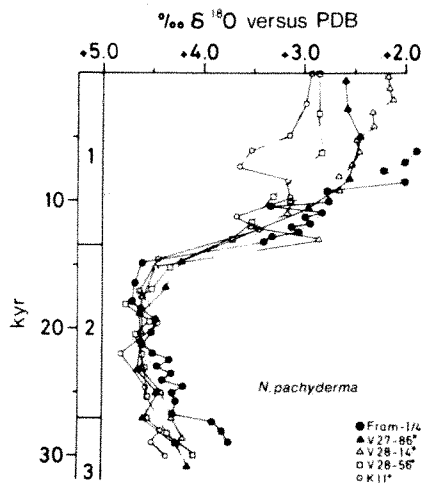


Fig. 3 O-isotope curves of cores from Norwegian-Greenland Sea[26,27] and of core Fram-I/4. A better comparison has been obtained by replotting the O-isotope data on a timescale defined by well-established radiocarbon ages[15,20-22].

hroughout all units, with smaller amounts of benthic fossils uch as foraminifers, sponges and ostracods. The sedimentologi- al and micropalaeontological data will be presented in more letail elsewhere[31].

The most important results and implications from the present tudy may be summarized as follows. (1) Sedimentation rates n the Arctic Ocean may be an order of magnitude higher than vas previously assumed. (2) Increased amounts of ice-rafted naterial accumulated during transitions towards both colder nd warmer climates, but during fully glacial and interglacial limates the supply of this component was strongly diminished. 3) The presence of planktonic foraminifers throughout both ores records a modest productivity of surface-subsurface vaters even during fully glacial conditions. (4) The reddish- rown colours of the sediments, as well as the benthic fossils ound throughout both cores, may be evidence of an efficient enewal of bottom water masses in the eastern Arctic Basin hroughout the late Quaternary. In contrast, however, the $\delta^{13}C$ ecords suggest a distinct change in oceanic circulation during ermination I towards present-day circulation patterns. (5) The bvious correspondence of the O-isotope records presented here vith those from low-latitude sediment cores may indicate an ctive and efficient exchange of Arctic Ocean water masses with hose of the global ocean throughout the late Quaternary. Fur- hermore, the close agreement in O-isotope data from our Arctic)cean cores and those from the Norwegian-Greenland ea[26,27] during stage 2 suggests similar hydrographies of surface- ubsurface waters in both deep-sea basins during fully glacial onditions.

Fram-I cores were collected under the supervision of Y. Kristoffersen. Isotope measurements were made at the ^{14}C aboratory of the University of Kiel. We thank M. Sarnthein, I. Erlenkeuser, G. Miller and N. Shackleton for discussions. his study was supported by the US Office of Naval Research, he Norwegian Polar Institute and the German Research Pro- ram on Climate.

eceived 1 November 1984; accepted 11 February 1985.

1. Donn, W. L. & Ewing, M. *Science* **152**, 1706-1712 (1966).
2. Donn, W. L. & Shaw, D. M. *Progr. Oceanogr.* **4**, 105-113 (1967).
3. Olausson, E. *Geol. För. Stockh. Förh.* **91**, 185-200 (1969).
4. Ewing, M. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 565-573 (Yale University Press, 1971).
5. Olausson, E. *Ambio spec. Rep.* **2**, 13-17 (1972).
6. Hughes, T., Denton, G. D. & Grosswald, M. G. *Nature* **266**, 596-602 (1977).
7. Herman, Y. & Hopkins, D. M. *Science* **209**, 557-562 (1980).
8. Clark, D. L. *Nature* **300**, 321-325 (1982).
9. Clark D. L., Whitman, R. R., Morgan, K. A. & Mackey, S. D. *Geol. Soc. Am. spec. Pap.* **181** (1980).
10. Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39-55 (1973).
11. Kristoffersen, Y. *Isdriftstasjonen Fram-I Ekspedisjonsrapport* (Norwegian Polar Institute, Oslo, 1979).
12. Lutze, G.-F. *et al. Init. Rep. DSDP* **47**, 727-739 (1979).
13. Ganssen, G. & Sarnthein, M. in *Coastal Upwelling: Its Sediment Record* (eds Suess, E. & Thiede, J.) 99-121 (Plenum, New York, 1983).
14. Broecker, W. S. & Van Donk, J. *Rev. Geophys. Space Phys.* **8**, 169 (1970).
15. Duplessy, J. C., Delibrias, G., Turon, J. L., Pujol, C. & Duprat, J. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **35**, 121-144 (1981).
16. Berglund, B. E. *Boreas* **8**, 98-118 (1979).
17. Duplessy, J. C. in *Climatic Change* (ed. Gribbin, J.) 46-67 (Cambridge University Press, 1978).
18. Craig, H. & Gordon, L. I. in *Stable Isotopes in Oceonographic Studies and Paleotemperatures* (ed. Tongiorgi, E.) 9-130 (Consiglio Nazionale delle Richerche, Laboratorio di Geologia Nucleare, Pisa, 1965).
19. Emiliani, C. & Shackleton, N. J. *Science* **183**, 511-514 (1974).
20. Koopmann, B. *ForschErgebn.* C**35**, 23-59 (1981).
21. Morley, J. J. & Hays, J. D. *Earth planet. Sci. Lett.* **53**, 279-295 (1981).
22. Sarnthein, M., Erlenkeuser, H. & Zahn, R. *Bull. Inst. Geol. Bassin d' Aquitaine* **31**, 393-407 (1982).
23. Ku, T. L. & Broecker, W. S. *Progr. Oceanogr.* **4**, 95-105 (1967).
24. Clark, D. L., Vincent, J.-S., Jones, G. A. & Morris, W. A. *Nature* **311**, 147-149 (1984).
25. Sejrup, H. P., Miller, G. H., Brigham-Grette, J., Lövlie, R. & Hopkins, D. *Nature* **310**, 772-775 (1984).
26. Kellogg, T. B., Duplessy, J. C. & Shackleton, N. J. *Boreas* **7**, 61-73 (1978).
27. Streeter, S. S., Belanger, P. E., Kellogg, T. B. & Duplessy, J. C. *Quat. Res.* **18**, 72-90 (1982).
28. Kroopnick, P. *Earth planet. Sci. Lett.* **49**, 469-484 (1980).
29. Sejrup, H. P., Jansen, E., Erlenkeuser, H. & Holtedahl, H. *Quat. Res.* **21**, 74-84 (1984).
30. Gammelsrod, T. & Rudels, B. *Polar Res.* **1**, 115-126 (1983).
31. Markussen, B., Zahn, R. & Thiede, J. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* (in the press).

Microhabitats of benthic foraminifera within deep-sea sediments

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Benthic foraminifera are protozoans found throughout the deep-sea environment, secreting a test of calcium carbonate or constructing a test of cemented sediment particles (agglutinated or arenaceous foraminifera). In typical deep-sea sediments, the organic cement of agglutinated taxa degrades upon burial in the sediment and, consequently, few specimens survive in the fossil record. In contrast, calcareous species are well preserved in most oceanic sediments, except at abyssal depths where most carbonate sediment is dissolved because of high levels of carbonate undersaturation of the bottom waters. Although benthic foraminifera have been widely used in studies of Cenozoic palaeoceanography, little is known about the ecology of deep-sea species. I present here an analysis of living (stained) benthic foraminifera within the upper 15 cm of deep-sea sediments, which reveals species-specific microhabitat preferences, with distinct morphological features found with epifaunal and infaunal species. The existence of infaunal habitats suggests that the distribution of certain foraminifera is not directly controlled by overlying bottom-water conditions, but by physicochemical conditions within the sediments. The microhabitat preferences may also explain interspecific carbon isotope differences, as existing data show that infaunal foraminifera generally have lower $\delta^{13}C$ isotope values than epifaunal species.

Early distributional studies demonstrated that deep-sea benthic foraminiferal assemblages are depth related[1] and this information was used in palaeoenvironmental studies of marine sedimentary sequences to estimate past water depths[2,3]. More recent studies have shown that deep-sea benthic foraminiferal faunal assemblages are related to the distribution of distinct water masses in the deep ocean, although the variables which control the faunal patterns are largely unknown[4]. In addition to faunal distribution studies, oxygen and carbon isotope analyses of benthic foraminifera have been used extensively to estimate polar ice volumes[5,6] and to study regional palaeocirculation conditions in the deep ocean[7-9].

A number of unanswered questions remain which limit the usefulness of the faunal and isotope data. Although significant correlations between bottom-water hydrographic variables (for

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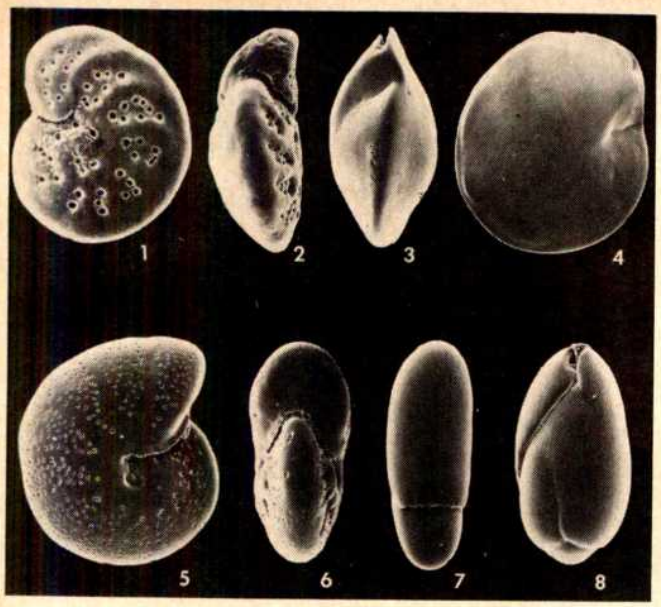


Fig. 1 Illustrations of important living deep-sea benthic foraminifera in OC-86/2-7-4. (1) *Planulina wuellerstorfi*, umbilical view (×103); (2) *P. wuellerstorfi*, edge view (×79). Pores can be seen on the umbilical side but are absent from the spiral side. (3) *Hoeglundina elegans*, edge view (×55); (4) *H. elegans*, umbilical view (×44); (5) *Melonis barleeanum*, side view (×82); (6) *M. barleeanum* edge view (×72); (7) *Chilostomella oolina* (×51); (8) *Globobulimina affinis* (×51). Small pores are present over most of the test surface of *C. oolina* and *G. affinis*. The epifaunal species (1–4) have plano-convex or biconvex shapes with surface pores on only one side or absent, whereas the infaunal species (5–8) have more rounded tests with surface pores evenly distributed over most of the test surface.

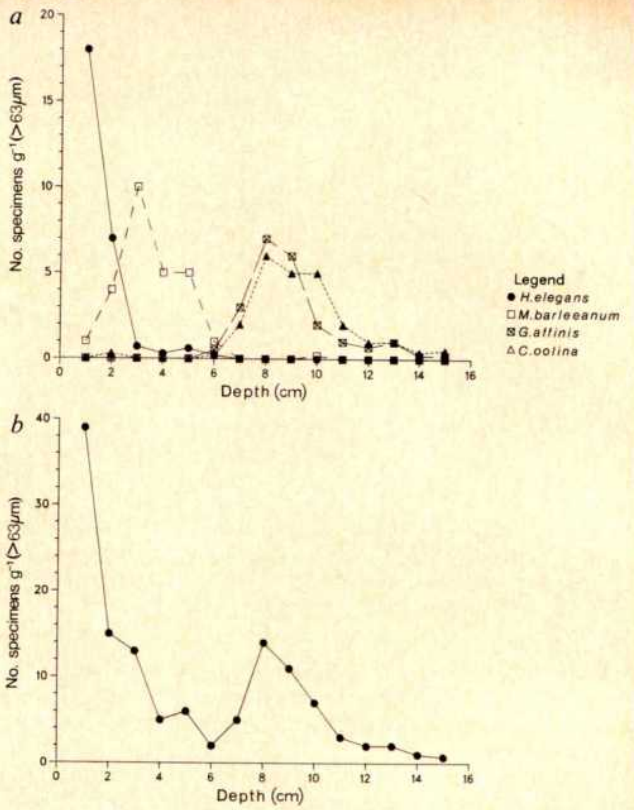


Fig. 2 Summary of benthic foraminiferal data from OC-86/2-7-4. *a*, Number of specimens (>150 μm) per g of >63-μm sediment of four dominant species; *b*, total number of specimens (>150 μm) per g of >63-μm sediment. The species are vertically stratified within the deep-sea sediments with a majority of the living fauna (73%) found below 2 cm.

example, temperature, salinity, dissolved oxygen) and faunal data do exist in regional studies, these correlations are not consistent over broad areas of the ocean, indicating that other variables are controlling faunal patterns. Analysis of the carbon isotope composition of benthic foraminiferal species shows that there are differences (frequently referred to as 'vital effects') between species, with some species secreting calcium carbonate in carbon isotope disequilibrium with the overlying bottom water[10–13]. Interspecific differences in $\delta^{13}C$ have led to speculation that certain species of deep-sea benthic foraminifera have infaunal microhabitats within the sediment where pore-water $\delta^{13}C$ influences carbonate $\delta^{13}C$ (refs 10–12). Palaeoceanographic studies often use time-series data from more than one species with disequilibrium factors applied to correct for species differences, with the assumption that these interspecific differences are constant through time. However, a number of studies have shown that for some species these fractionation factors are variable in the Cenozoic record[14,15].

To gain insight into these questions, two box cores were taken from the western North Atlantic on R/V *Oceanus* cruise 86, leg 2 (OC-86/2), in September 1980, using an Ocean Instruments Mark III box corer (0.25-m^2 surface area) divided into 25 10-cm × 10-cm subsamples. OC-86/2 box core 7-4 (OC-86/2-7-4) was taken at 38°12.9′N, 71°30.4′W in 3,000-m water depth on the eastern US continental rise and *Oceanus* 86/2 box core 5-3 was taken at 35°22.8′N, 65°20′W in 4,800-m water depth on the Bermuda Rise. Both box cores had clear water above the surface indicating the surface sediments were undisturbed during the coring process. Slices (1 cm thick) of two subsamples from OC-86/2-7-4 were taken from the sediment/water interface to 15 cm depth, and from one subsample in OC-86/2-5-3 to 9 cm depth. Each 100-cm^3 sample was preserved in 150 ml of buffered 4% formalin solution and the samples were treated with Rose Bengal stain to identify benthic foraminifera living at the time of collection. In the laboratory, each sample was washed over a 63-μm sieve, the >63-μm fraction was dried, weighed and dry sieved over a 150-μm sieve. All of the living (stained) benthonic foraminifera were picked from the >150-μm sample, sorted by species and counted. Rose Bengal is a biological stain that absorbs on the surface of protoplasm and has been widely used in studies of living benthic foraminifera. This method does have limitations (see ref. 16). A living individual is recognized by the presence of a bright red or violet coloration, within at least one chamber of the test, that can be seen in species with a hyaline test wall. Agglutinated foraminifera with test walls composed of cemented particles or miliolids that have an opaque porcellaneous calcareous wall were not considered here because it was difficult to observe stained protoplasm within the interior portion of the test. Some specimens had a tint of pink colour owing to the presence of an organic membrane, or spots of red colour reflecting particles of organic matter, but these were not counted as living specimens.

The dominant living species in one subsample of core 7-4 are illustrated in Fig. 1 and plotted in Fig. 2*a*, where the data are expressed as the number of living specimens (>150 μm) per gram of >63-μm dry sediment (Table 1). (Results from a second subsample are almost identical and are not presented here.) In the top 2 cm of sediment, *Hoeglundina elegans* (d'Orbigny) is the dominant species and is rare at 2–5 cm. Additional species within the top 1 cm include *Cibicidoides kullenbergi* (Parker), *Oridorsalis tener* (Brady) and *Planulina wuellerstorfi* (Schwager). Within the 1–6-cm interval, *Melonis barleeanum* (Williamson) is the dominant species with a maximum of 8 specimens per g in the 2–3-cm interval. From 6–7 cm to the bottom sample (14–15 cm), two species, *Globobulimina affinis* (d'Orbigny) and *Chilostomella oolina* (Schwager), are dominant, reaching maxima of 6–7 specimens per g in the 7–9-cm interval. The total number of living specimens per gram of >63-μm sediment (Fig.

Table 1 Benthic foraminiferal data from OC-86/2-7-4

Interval (cm)	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15
Species															
Chilostomella oolina	-	1	-	-	-	1	6	29	26	20	9	4	5	2	2
Cibicidoides kullenbergi	6	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Cibicidoides spp.	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Globobulimina affinis	-	-	1	-	-	2	12	33	29	10	5	3	5	1	1
Hoeglundina elegans	29	20	2	1	2	1	-	-	-	-	-	-	-	-	-
Melonis barleeanum	2	12	30	18	17	5	-	-	-	1	-	-	-	-	-
Melonis pompilioides	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Oridorsalis tener	10	5	1	-	-	-	-	-	-	-	-	-	-	1	-
Parafissurina sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Planulina wuellerstorfi	10	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Pullenia simplex	-	3	3	-	1	-	-	1	-	-	-	-	-	-	-
Robertina bradyi	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Uvigerina sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown	-	-	-	1	-	-	-	2	-	1	-	-	-	-	-
Total no.	64	45	39	20	20	9	18	65	55	32	14	7	10	4	3
Weight, >63 μm fraction (g)	1.64	2.97	3.00	3.64	3.15	4.39	3.70	4.56	5.11	4.34	4.20	4.25	4.52	3.90	4.26

The number of specimens (>150 μm) of each species, the total number of specimens and the weight of the >63-μm fraction examined are shown for each sample interval.

2*b*) shows a maximum of 39 specimens per g in the 0-1-cm interval and a secondary maximum of 14 specimens per g at the 7-8-cm interval. The faunal data reveal that 73% of the living faunal assemblage was below 2 cm in OC-86/2-7-4. The presence of living benthic foraminifera at depth was verified by picking a few specimens of *G. affinis* from a wet subsample, dissolving the test in a 0.01 M EDTA solution and observing the stained protoplasm. The results from OC-86/2-7-4 differ from OC-86/2-5-3 data, which show that the majority of the living fauna (84%) are confined to the 0-1-cm interval. The number of living specimens per gram in the 0-1-cm interval is 13, with *Epistominella umbonifera* (Cushman) the dominant species, and 1 specimen per g in the 1-2-cm interval. Four specimens were found scattered within the 2-9-cm interval. The data from the two cores show that foraminiferal distributions within the sediments vary with water depth.

The foraminifera are vertically stratified in OC-86/2-7-4, with species-specific microhabitat preferences within the upper 15 cm of sediment. An infaunal habitat for calcareous benthic foraminifera within deep-sea sediments has not been noted previously, although it has been documented for some shallow-water calcareous species[17-24]. Based on the OC-86/2 data, *M. barleeanum*, *C. oolina* and *G. affinis* have infaunal microhabitat preferences. The species confined largely to the top 1 cm, *P. wuellerstorfi*, *C. kullenbergi* and *O. tener*, are more likely to have an epifaunal preference, although these species may be stratified within the 0-1-cm interval. A sampling interval of <1 cm is necessary to determine whether vertical stratification occurs within this interval. *H. elegans* is found throughout the top 2 cm, indicating that this species may have both an epifaunal and infaunal microhabitat, or that its distribution may be influenced by bioturbation moving living species downward from the surface. Bioturbation cannot be used, however, to explain the distribution of species below 2 cm, as *C. oolina* and *G. affinis* are not found in the 0-1-cm interval and these species and *M. barleeanum* have subsurface maxima.

A correlation between benthic foraminiferal morphology and environment was noted by Bandy[1] in continental margin surface sediments; surface area and ornamentation of a number of species were shown to vary with water depth. Although several phenotype studies of benthic foraminifera have followed these initial observations, little is known about the functional adaptive morphology of the benthic foraminiferal test. The test morphologies of the benthic foraminifera in OC-86/2-7-4 show a distinct pattern with sediment depth (Fig. 1). Species found in the 0-2-cm interval, *H. elegans*, *P. wuellerstorfi*, *O. tener*, *C. kullenbergi* and *Cibicidoides* spp., have plano-convex or biconvex shapes, with wall pores found only on the spiral side or completely absent from the entire test. Of species found living below 2 cm, *M. barleeanum* has planispiral coiling with a rounded periphery, *C. oolina* is planispiral and cylinder shaped and *G. affinis* has triserially arranged chambers with a globular to ovate test. In contrast to the species found in the upper 2 cm, the dominant subsurface species have pores evenly distributed over most of the test.

The correlations between test shape, pore distribution and sediment depth suggest that these morphological variables are related to the microhabitat preference of the individual species. The plano-convex or biconvex shapes of the surface species may be advantageous for attachment to the bottom during times of bottom turbulence and for stability in traveling on or near the surface. For example, in shallow-water turbulent environments, *Cibicides* spp., which have plano-convex shapes, are attached to the substrate to resist transport or destruction in these high-energy regions. The plano-convex or biconvex shapes that would be advantageous for stability and mobility at or near the surface would be unnecessary within the sediment and these shapes are indeed absent.

Leutenegger and Hansen[25] noted that a number of species from low-oxygen (<0.3 ml l^{-1} O_2) environments, including *Globobulimina pacifica*, a species closely related to *G. affinis*, had a lower density of mitochondria that were concentrated near pore openings within the cell, than specimens from higher-oxygen environments that had concentrations of mitochondria more evenly distributed throughout the cell. The concentration of mitochondria below the pore entrances was suggested to increase the diffusion of oxygen into the cell in low-oxygen environments. It was also found that an osmiophilic organic lining of *G. pacifica* did not cover the pore entrance, which was interpreted to allow a faster rate of molecular transport in low-oxygen environments. These observations support earlier conclusions[16] that pores are important for gas exchange in foraminifera.

If gas-exchange morphological adaptations are significant, the distribution and density of pores should be distinctly different in epifaunal and infaunal species, because the microenvironments in which they live would be expected to have significantly different dissolved-oxygen levels. The infaunal species (*M. barleeanum*, *C. oolina*, *G. affinis*) have pores evenly distributed over most of the test surface, which should be desirable for enhancing gas exchange in a low-oxygen environment. The pores found in *Cibicidoides* spp. and *P. wuellerstorfi* are found only on the spiral side and may be for protoplasmic streaming as well as for gas exchange, as these openings are much larger (~10 μm) than those found in other species (~1 μm). One species, *E. umbonifera*, which is found in the 0-1-cm interval of OC-86/2-5-3, is different from the other trochospiral species in that it has pores on both sides of the test. The foraminiferal test shapes

may reflect adaptations promoting gas exchange in low-oxygen environments within the sediment as well, as the shapes of species with subsurface maxima have higher surface area/volume ratios than the epifaunal species.

The distribution of live benthic foraminifera within these deep-sea sediments suggests that certain foraminifera with infaunal microhabitats are not directly controlled by overlying bottom-water conditions, but by physicochemical conditions within the sediment, as this environment is geochemically different from the sediment/water interface[26-28]. Some species do not appear to be confined to infaunal habitats, but instead are responding to a set of physicochemical variables independent of sediment depth. For example, *Chilostomella* and *Globobulimina* are found at depth in OC-86/2-7-4, but related species are common in California Borderland surface sediments (based on Rose Bengal stained data) associated with bottom water dissolved-oxygen values of <3 ml l^{-1} and generally <1 ml l^{-1} (ref. 29). The depth distribution of a particular species probably varies regionally in response to different physicochemical conditions within the sediments, ontogenetic differences in microhabitat requirements, or as a result of seasonal cycles in the deep sea.

Recent carbon-isotope data from pore waters in the upper few centimetres of Pacific Ocean and Atlantic Ocean sediments (ref. 30 and F. L. Sayles and W. B. Curry, in preparation) indicate that a $\delta^{13}C$ gradient of $\sim 1‰$ exists in the upper one to a few centimetres. The $\delta^{13}C$ values decrease with depth in the sediment because of the oxidation of ^{12}C-enriched organic matter, which results in a decrease in the $\delta^{13}C$ of the pore waters. The demonstration from the OC-86/2 data that a number of deep-sea benthic foraminifera do have infaunal microhabitats, together with the existence of $\delta^{13}C$ gradients within deep-sea sediments, indicate that the $\delta^{13}C$ disequilibrium of some benthic foraminifera can probably be explained in part by the $\delta^{13}C$ composition of the pore waters. Benthic foraminiferal carbon-isotope data[10,11,13,31] show that plano-convex or biconvex forms, inferred to be epifaunal species, are generally nearest to isotope equilibrium, whereas species with shapes suggesting infaunal habitats are depleted in ^{13}C. For example, a species of *Globobulimina* was highly variable with $\delta^{13}C$ up to 2-3‰ below equilibrium of the overlying bottom water in the California Borderlands[12]. One notable exception to this pattern, however, is *O. tener*, which has consistently lighter values than other biconvex forms. The existing data suggest that a relationship exists between carbon isotope composition and test morphology, which would be expected if the pore-water geochemistry of the microenvironment influences the benthic foraminiferal carbon-isotope data.

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1. Bandy, O. L. *21st Int. geol. Congr.*, Copenhagen, Pt 22, 7-25 (1960).
2. Barr, F. T. & Berggren, W. A. in *The Geology of Libya* (eds Salem, M. J. & Busrewil, M. T.) 163-192 (Academic, New York, 1981).
3. Berggren, W. A. & Aubert, J. *Geol. Surv. prof. Pap.* **1213**, 4-21 (1983).
4. Douglas, R. G. & Woodruff, F. in *The Sea* Vol. 7 (ed. Emiliani, C.) 1233-1327 (Wiley-Interscience, New York, 1981).
5. Shackleton, N. J. & Opdyke, N. D. *Quat. Res.* **3**, 39-55 (1973).
6. Shackleton, N. J. & Opdyke, N. D. *Geol. Soc. Am. Mem.* **145** (eds Cline, R. M. & Hays, J. D.) 449-464 (1976).
7. Boyle, E. A. & Keigwin, L. D. Jr *Science* **218**, 784-787 (1982).
8. Curry, W. B. & Lohmann, G. P. *Nature* **306**, 577-580 (1983).
9. Shackleton, N. J., Imbrie, J. & Hall, M. A. *Earth planet. Sci. Lett.* **65**, 233-244 (1983).
10. Woodruff, F., Savin, S. M. & Douglas, R. G. *Mar. Micropaleont.* **5**, 3-11 (1980).
11. Belanger, P. E., Curry, W. B. & Matthews, R. K. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **33**, 205-220 (1981).
12. Grossman, E. L., *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **47**, 301-327 (1984).
13. Graham, D. W., Corliss, B. H., Bender, M. L. & Keigwin, L. D. Jr *Mar. Micropaleont.* **6**, 483-497 (1981).
14. Savin, S. M. *et al. Mar. Micropaleont.* **6**, 423-450 (1981).
15. Keigwin, L. D. Jr *Init. Rep. DSDP* **68**, 445-453 (1982).
16. Boltovskoy, E. & Wright, R. *Recent Foraminifera*, 1-515 (Junk, The Hague, 1976).
17. Boltovskoy, E. *Contr. Cushman Fdn Foraminifera Res.* **17**, 43-45 (1966).
18. Brooks, A. L. *Limnol. Oceanogr.* **12**, 667-684 (1967).
19. Schafer, C. T. *Limnol. Oceanogr.* **16**, 944-951 (1971).
20. Ellison, R. L. *Geol. Soc. Am. Mem.* **133**, 247-262 (1972).
21. Matera, N. J. & Lee, J. J. *Mar. Biol.* **14**, 89-103 (1972).
22. Frankel, L. *J. Paleont.* **46**, 62-65 (1972).
23. Frankel, L. *J. Paleont.* **49**, 563-565 (1975).
24. Ellison, R. L. & Peck, G. E., *J. Foram. Res.* **13** (4), 231-241 (1983).
25. Leutenegger, S. & Hansen, H. J. *Mar. Biol.* **54**, 11-16 (1979).
26. Froelich, P. N. *et al. Geochim. cosmochim. Acta* **43**, 1075-1090 (1979).
27. Berner, R. R. in *The Environment of the Deep Sea* (eds Ernst, W. G. & Morin, J. G.) 154-176 (Prentice-Hall, Hemel Hempstead, 1982).
28. Sayles, F. L. *Geochim. cosmochim. Acta* **45**, 1061-1086 (1981).
29. Douglas, R. G. & Heitman, H. L. *SEPM spec. Publ.* **27**, 231-246 (1979).
30. McCorckle, D. C. & Emerson, S. R. *EOS* **64**, 721 (1983).
31. Shackleton, N. J., Hall, M. A. & Boersma, A. *Init. Rep. DSDP.* **74**, 599-612 (1984).

Weak neutral currents and the origin of biomolecular chirality

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It has long been known that Earth's biochemistry is overwhelmingly dissymmetric or chiral[1-4]. In model chemical systems[5-7] that spontaneously evolve to a state dominated by either the L or the D enantiomer, parity violation in β-decay and that attributable to weak neutral currents (WNC) in molecules[8,9] is thought to be too small to have any significant influence on the emergent chirality[10,11]. Other conceivable systematic chiral influences are generally even weaker[12-14]. We show here that there is a simple and extremely sensitive mechanism by which a minute but systematic chiral interaction, no stronger than the WNC interaction in amino acids, can, over a period of ~15,000 yr, determine which enantiomer will dominate. Such a mechanism is especially interesting when considering the origins of terrestrial biochemistry, particularly in view of the work by Mason and Tranter[15], who found that it is the terrestrially dominant L amino acids that are favoured by the WNC interaction.

The process occurs in a randomly fluctuating environment. Chemical systems that, in conditions that are thermodynamically far from equilibrium, can evolve spontaneously to a chirally asymmetric state—that is 'break chiral symmetry'—exhibit a universal behaviour that is a consequence of the symmetry properties of the system[14,16-18]. The amplitude α of the chiral dissymmetry, with the inclusion of fluctuations, obeys the stochastic (Langevin) equation

$$\frac{d\alpha}{dt} = -A\alpha^3 + B(\lambda - \lambda_c)\alpha + Cg + C'\eta f_2(t) + \varepsilon_1^{1/2} f_1(t) \quad (1)$$

in which A, B and C are constants that depend on the kinetics[14,16]; $g \equiv (\Delta E/kT)$, where k is the Boltzmann constant and T the temperature, is the factor by which the Arrhenius reaction rate constants for the L and the D enantiomers differ because of a small difference, ΔE, in their reaction barrier energies caused by an extremely weak chiral interaction[14]—such as WNC. In addition to such systematic effects, there is the fluctuating chiral influence from the environment, such as that attributable to circularly polarized ultraviolet light, represented by $C'\eta f_2(t)$, where $f_2(t)$ is assumed to be a normalized gaussian white noise. We estimate the numerical value of $C'\eta$ from the best known data on circularly polarized light. The intrinsic

thermodynamic fluctuations[19,20] are represented by $\varepsilon_1^{1/2} f_1(t)$; again $f_1(t)$ is assumed to be a normalized gaussian white noise; $\varepsilon_1 = (Q/VN_A)$, where Q can be calculated from the chemical kinetics; V is the volume over which the concentration of the reactants may be assumed homogeneous and N_A is the Avogadro number. The most general stochastic equation must include fluctuations in A and B but, as they have no significant effect on the mechanism[21,22], we ignore them.

As an example and for later numerical considerations, we shall consider the following model scheme of reactions studied in detail in ref. 14

$$S + T \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_{L(D)} \qquad \text{(i)}$$

$$S + T + X_{L(D)} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} 2X_{L(D)} \qquad \text{(ii)}$$

$$X_L + X_D \xrightarrow{k_3} P. \qquad \text{(iii)}$$

In this scheme, the chiral species X in the two enantiomeric forms X_L and X_D is produced from the achiral substrate S and T directly through reaction (i) and autocatalytically through reactions (ii). With a suitable supply of S and T (to maintain their concentrations at a fixed level), and the irreversible removal of X through reaction (iii), the system can be driven far from thermodynamic equilibrium. The variables of equation (1) for this system are: $\lambda \equiv [S][T]$ and $\alpha \equiv ([X_L]-[X_D])/2$ (where [] denote concentrations). When λ exceeds a certain critical value λ_c, the steady-state value of α switches from zero to either $\alpha > 0$ or $\alpha < 0$ (ref. 14). If the small chiral interaction influences reactions (i) and (ii) so that the rate constants of the L and D enantiomers are unequal, $k_{1L} = k_{1D}(1+g)$ and $k_{2L} = k_{2D}(1+g)$, then $C = [k_1 + k_2\beta_c]\lambda_c$, where $\beta \equiv ([X_L]+[X_D])/2$, k_1 and k_2 are rate constants when $g = 0$, and the subscript 'c' denotes values at the critical point. Similar, but more involved, expressions may be obtained for A and B (ref.14).

In contrast to the earlier studies[23-25] that mainly examined such systems for $\lambda > \lambda_c$, we study the system as it slowly evolves through the critical point. The macroscopic steady states (when the fluctuations are ignored) and a sample fluctuating trajectory for the time evolution of α of equation (1) are shown in Fig. 1. When λ is well below λ_c, there is only one steady state, $\alpha \simeq Cg/B(\lambda - \lambda_c) \ll 1$, which becomes unstable when λ goes beyond λ_c; for $\lambda > \lambda_c$, the system has two new supercritical branches, $\alpha \simeq \pm\sqrt{B(\lambda - \lambda_c)/A}$, to which it can evolve. In the absence of g, the two supercritical branches emerge symmetrically (as a parabola) from the point λ_c and, as the system evolves through the critical point, the fluctuations make both states equally probable. When $g \neq 0$ this is no longer true; one branch is favoured. The effect of g is most marked in the vicinity of the critical point where it separates the two stable supercritical branches by a minimum of $S = (3/2)(4/A)^{1/3}(Cg)^{1/3}$; in contrast, well above and below the critical point the shift is proportional to Cg. As $Cg \ll 1$, the fractional exponent indicates the enhanced sensitivity of the system near the critical point. However, in this region the α fluctuations are also large. Our aim is to obtain the probability of the system evolving to the favoured branch as λ goes through the critical point at a given rate.

An important factor in calculating this probability is $\varepsilon_1 = Q/VN_A$, which is derived assuming the system is homogeneous over the volume V. We estimate V by considering the homogenization that occurs through diffusion and, for prebiotic considerations, large-scale mixing as might occur in an ocean or a lagoon.

The evolution of the system may be considered in four stages (see Fig. 1). In stages I and IV, the system is well below and well above the critical point respectively; during stages II and III, it is in the vicinity of the critical point where the selection of branches takes place. In stages I and II, there is only one stable steady state and hence the system can be homogeneous over limitlessly large volume. In stage II, if $\alpha \ll 1$ and $\lambda \simeq \lambda_c$, α

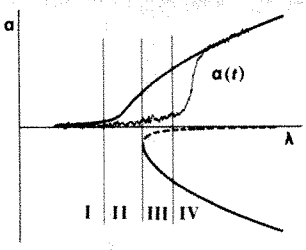


Fig. 1 A sample trajectory of α in equation (1) as λ increases through the critical point (fluctuations exaggerated). Solid line, stable steady states; dashed line, unstable steady state. Stages I–IV are explained in the text.

begins to grow slowly, essentially at an average rate Cg. In stage III, however, the system has two possible steady states and hence the volume over which it may be assumed homogeneous has an upper limit. In this stage, in a small volume δV, the autocatalysis of the chemistry (reflected in the term $B(\lambda - \lambda_c)$, $\lambda > \lambda_c$) will cause a fluctuation in α to grow; this growth, however, is curtailed by diffusion and large-scale mixing which transport the excess of α out of δV. As the rate of growth of α due to the chemical reactions is proportional to δV, whereas depletion is proportional to the surface area of δV, there is a 'nucleation volume' V_c below which α cannot grow. Within this volume V_c, homogeneity is maintained. Diffusion alone can maintain homogeneity over a length scale $l_D \simeq \sqrt{D/B(\lambda - \lambda_c)}$, D being the diffusion coefficient[26,27]. In our numerical simulation, in stage III, $B(\lambda - \lambda_c) \leq 10^{-11}\ s^{-1}$, and with $D \sim 10^{-5}\ cm^2\ s^{-1}$ we see that $\lambda_D \sim 10^3$ cm. Also, stages II and III are traversed in ~6,000 yr, during which we may expect homogenization due to large-scale mixing to be on an oceanic scale. We assume this volume V_c to be at least 4×10^9 l (1 km × 1 km × 4 m) for our estimate of ε_1. As indicated by the fluctuating trajectory in Fig. 1, by the time stage IV is reached, if the system is already well within the region of attraction of the favoured (upper) branch, it becomes increasingly improbable for a fluctuation in α, occurring at least over a volume V_c, to be large enough to reach the unfavoured (lower) branch.

To obtain the probability for the selection of branches, we study the Fokker–Planck[28,29] equation associated with equation (1), which describes the evolution of the probability density $P(\alpha, t)$ of α:

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial \alpha}(-A\alpha^3 + B(\lambda(t) - \lambda_c)\alpha + Cg)P(\alpha, t) + \left(\frac{\varepsilon}{2}\right)\frac{\partial^2}{\partial \alpha^2}P(\alpha, t) \qquad (2)$$

where $\varepsilon = \varepsilon_1 + (C'\eta)^2$. We suppose, at $t = 0$, λ is well below the critical point and is gradually increasing. Fluctuations in λ have an insignificant role in the process of selection[21], so we ignore them in our theoretical discussion, though not in our numerical simulation of the above model shown in Fig. 2. From equation (2) it follows that, for λ well below λ_c, $P(\alpha, t)$ is essentially a gaussian whose centre is at $\bar{\alpha} \simeq Cg/B(\lambda_c - \lambda)$, very close to zero (stage I in Fig. 1). As λ increases at a reasonable rate ($\sim 10^{-5}\ M^2\ (10^4\ yr)^{-1}$ for the model) in the vicinity of the critical point, $P(\alpha, t)$ is still a gaussian, but it begins to relax very slowly to the stationary distribution. Thus, in this stage (stages II and III in Fig. 1), $P(\alpha, t)$ is vanishingly small for large α and hence the $-A\alpha^3$ term may be neglected in comparison with the other terms.

For selection, then, we need only consider

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial \alpha}(B(\lambda(t) - \lambda_c)\alpha + Cg)P(\alpha, t) + \left(\frac{\varepsilon}{2}\right)\frac{\partial^2}{\partial \alpha^2}P(\alpha, t) \qquad (3)$$

When $\lambda \simeq \lambda_c$, the term $B(\lambda - \lambda_c)\alpha$, for $\alpha \ll 1$, also becomes small compared with Cg. Here we have a gaussian whose peak is shifting at a constant rate Cg, but whose width is also increasing

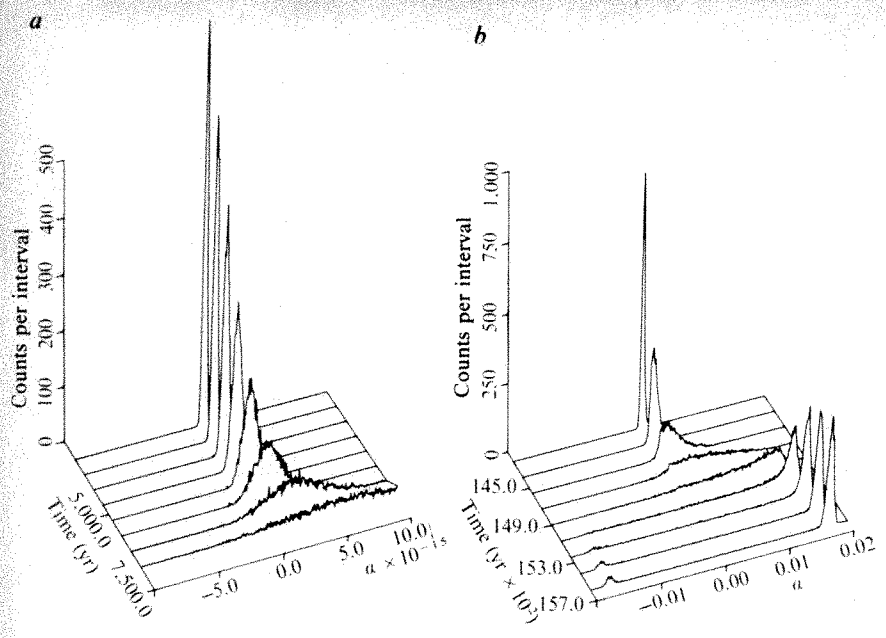


Fig. 2 Results of simulation of equation (2) by 5,000 sample trajectories of equation (1), with $\lambda(t) = \lambda_0 + \gamma t + 0.05 \times \lambda_c \times f(t)$, where $\lambda_0 = 0.8 \times 10^{-5}\ M^2$, $\gamma = 3.171 \times 10^{-17}\ M^2\ s^{-1}$, and $f(t)$ is normalized gaussian noise; t goes from 0 to 15,600 yr. Other parameters are as given in the text. Each curve is a histogram of the number of times $\alpha(t)$ fell within an interval 1/400 of full α scale for the given t. *a*, $P(\alpha)$ drifting and spreading while λ is near λ_c, which here occurs at $t = 3{,}000$ yr. *b*, $P(\alpha)$ spreading and splitting for $\lambda \gg \lambda_c$. Note the change of scale in α by 10^{12} between *a* and *b*, because of which the number of trajectories per interval has sharply increased in *b*.

because of the 'diffusion term' containing ε. In a time interval T, the drift of the peak will be CgT, while the increase in the width will be $\sqrt{\varepsilon T}$. Thus, for sufficiently large T, even when $Cg \ll \sqrt{\varepsilon}$, CgT can exceed $\sqrt{\varepsilon T}$, which implies that much of $P(\alpha, t)$ will drift into the region $\alpha > 0$ (for $Cg > 0$). This is the heart of the selection mechanism. Now, as λ goes beyond λ_c (stage IV in Fig. 1), the term $B(\lambda - \lambda_c)$ is positive and will cause $P(\alpha, t)$ to spread out rapidly on either side. $P(\alpha, t)$, thus split into two parts, will accumulate around the macroscopic steady states because of the term $-A\alpha^3$; note, however, that by the time this term becomes important, how much of $P(\alpha, t)$ will have evolved into $\alpha > 0$ and $\alpha < 0$ regions will already have been determined. Thus, equation (3) is all we need to calculate the probability of branch selection—an approximation well supported by the numerical solution of the complete equation.

The solution to equation (3), well known[29,30] for constant λ, can be extended to time-dependent λ. With $P(\alpha,0) = \delta(\alpha - \alpha_0)$, the solution $P(\alpha, t|\alpha_0)$ is a gaussian that is drifting and spreading

$$P(\alpha, t|\alpha_0) = \frac{1}{[\pi z(t)]^{1/2}} \exp\left[\frac{(\alpha - \bar{\alpha}(t))^2}{z(t)}\right] \tag{4}$$

where

$$\bar{\alpha} = \alpha_0 \exp(w(t)) + Cg \exp w(t) \int_0^t \exp(-w(t'))\mathrm{d}t'$$

in which

$$w(t) = \int_0^t B(\lambda(t') - \lambda_c)\,\mathrm{d}t'$$

and

$$z(t) = 2\varepsilon \int_0^t \exp\left(2\int_{t'}^t B(\lambda(t'') - \lambda_c)\,\mathrm{d}t''\right)\mathrm{d}t'$$

For the probability of selection of the $\alpha > 0$ chiral steady state, P_+, we have: $P_+ = \mathrm{Lt}_{t\to\infty} \int_0^\infty P(\alpha, t/\alpha_0)\,\mathrm{d}\alpha$. Because $P(\alpha, t|\alpha_0)$ is a gaussian and $\alpha_0 \approx 0$, we may write

$$P_+(t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{N} \mathrm{e}^{-x^2/2}\,\mathrm{d}x \tag{5}$$

where

$$N = Cg\left[\exp w(t) \int_0^t \exp(-w(t'))\,\mathrm{d}t'\right] \Big/ \sqrt{z(t)/2} \tag{6}$$

gives the number of standard deviations by which the peak of $P(\alpha, t)$ has drifted from the origin. Now, if we let $\lambda = \lambda_0 + \gamma t$ (λ_0 well below λ_c), for $t \to \infty$, we get

$$N = Cg(\varepsilon/2)^{-1/2}(B\gamma/\pi)^{-1/4} \tag{7}$$

Thus, equations (7) and (5) give the required probability for the selection of a branch resulting from a chiral interaction.

For the biomolecular context we consider the above model with kinetic constants $k_1 = 5 \times 10^{-5}\ M^{-1}\ s^{-1}$, $k_2 = 2.5 \times 10^{-5}\ M^{-2}\ s^{-1}$, $k_3 = 10^{-3}\ M^{-1}\ s^{-1}$, $k_{-1} = 2.5 \times 10^{-10}\ s^{-1}$ and $k_{-2} = 1.25 \times 10^{-10}\ M^{-1}\ s^{-1}$. With these values the coefficients of equation (1) are: $A = 1.7 \times 10^{-7}\ M^{-2}\ s^{-1}$, $B = 2.5 \times 10^{-5} M^{-2}\ s^{-1}$, $C = 2.5 \times 10^{-10}\ M\ s^{-1}$ and $\lambda_c = 1.0 \times 10^{-5}\ M^2$; all concentrations are $\sim 10^{-3}$ M or less during the process of selection and $[X] \sim 10^{-2}$ M well above λ_c. If we suppose [S] and [T] are increasing slowly so that $\lambda \equiv [S][T]$ increases from $0.5\,\lambda_c$ to $1.5\,\lambda_c$ in 10,000 yr, then $\gamma = 3.2 \times 10^{-17}\ M^2\ s^{-1}$. For $\varepsilon_1 = Q/VN_A$, $Q = (k_1\lambda_c)/2$ for the model [14], and we take $V = 4 \times 10^9$ l as explained above, so that $\varepsilon_1 \simeq 10^{-43}\ M^2\ s^{-1}$. Using the results of Mason and Tranter for WNC[15], we take $g = 10^{-17}$, which makes $Cg = 2.5 \times 10^{-27}$. For the magnitude of the random chiral influences, we take $C'\eta = 3 \times 10^{-22}$, an estimate explained below. Using these values in equation (7) we get $N \simeq 2.0$, which implies $P_+ \simeq 0.98$, a 98% chance that the enantiomer favoured by WNC will emerge dominant even though the r.m.s. values of the random chiral influences are five orders of magnitude larger. Such sensitivity cannot be realized if the system does not evolve through the critical point.

To check the validity of our approximation of using equation (3) instead of equation (2), we numerically modelled the stochastic equation (1), with $\lambda = \lambda_0 + \gamma t + 0.05\,\lambda_c f(t)$, $f(t)$ being a gaussian noise. The results are shown in Fig. 2. For easier graphical visualization, we set $\varepsilon = 3.9 \times 10^{-43}$. Then, according to equation (5), $N = \sqrt{2}$, implying $P_+ = 0.921$ selectivity. Figure 2 is the result of averaging 5,000 trajectories and gives a $P_+ = 0.916$ in good agreement with the analytical result. If λ evolves extremely slowly, the approximation of using equation (3) is not valid. In the limit of infinitely slow λ_D, the results in refs 14, 16 can be used; for the intermediate region no analytical results exist.

For the estimate of the r.m.s. value of the random environmental factors, we take circularly polarized light in the ultraviolet range to be typical. (Hardly any reliable data exist for other effects.) Chirally selective photochemical effects of circularly polarized light are well known[31–33]. In our model, let us assume that chirally selective degradation of X (back reaction of reaction

(i)), at a rate $k'[X]$, occurs with a mean life of about 3,000 yr, that is $k' \simeq 10^{-11}\,s^{-1}$ for the average solar intensity. (Note, this is also the racemization rate.) For 100% circularly polarized light, the rate of decay of one of the enantiomers is faster by a factor $s \simeq 10^{-3}$ or less for most molecules[32,33], although there are exceptions[34]. Only a fraction $q \simeq 10^{-3}$ of the solar intensity at dawn and dusk is found to be circularly polarized in the infrared frequencies and is at least an order of magnitude smaller for the ultraviolet[35]. The sense of polarization depends on the direction and on the average it is zero. Considering the reduction of q for daylight intensities and attenuating factors in a large body of water, we may take $q_{rms} \simeq 10^{-6}$ with a correlation time $\tau \simeq 10^3$ s. On the evolutionary timescale of 10^{10}–10^{12} s considered here, this may be considered white noise of strength $\sqrt{2\tau}\, q_{rms}$ (equivalent to diffusion constant of $(1/2\tau)$ steps per unit time, each of length $2\tau q_{rms}$). Thus, the rate of asymmetric synthesis is $k'[X_{L(D)}]qs$; when q is a fluctuating quantity it may be represented by a gaussian white-noise term $C'\eta f_2(t)$, with $C'\eta = k'[([X_L]+[X_D])/2]s\sqrt{2\tau}\, q_{rms}$. By analogy with Cg, we may define $C' \equiv k'([X_L]+[X_D])/2$ and $\eta = s\sqrt{2\tau}\, q_{rms}$. With the above numerical values and $[X] \simeq 10^{-3}$ M, $C'\eta \simeq 3 \times 10^{-22}$, the value used in our numerical estimates.

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1. Mason, S. F. *Int. Rev. phys. Chem.* **3**, 217–241 (1983); *Nature* **311**, 19–23 (1984).
2. Elias, W. E. *J. chem. Educ.* **49**, 448–454 (1972).
3. Miller, S. W. & Orgel, L. E. *The Origin of Life on Earth* (Prentice-Hall, New Jersey, 1974).
4. Fox, S. W. & Dose, K. *Molecular Evolution and the Origin of Life* (Dekker, New York, 1977).
5. Frank, F. C. *Biochim. biophys. Acta* **11**, 459–463 (1953).
6. Seelig, F. F. *J. theor. Biol.* **31**, 335–361 (1971).
7. Decker, P. *J. molec. Evol.* **4**, 49–65 (1974).
8. Zel'dovich, Ya. B., Saakyan, D. B. & Sobel'man, I. I. *Pis'ma Zh. eksp. teor. Fiz.* **25**, 106–109 (1977) (*J. exp. theor. phys. Lett.* **25**, 94–98).
9. Hegstrom, R. A., Rein, D. W. & Sandars, P. G. H. *J. chem. Phys.* **73**, 2329–41 (1980).
10. Thiemann, W. (ed.) *Origins Life* **11**, 1–194 (1981).
11. Keszthelyi, L. *Origins Life* **14**, 375–382 (1984).
12. Mead, C. A. & Moscowitz, A. *J. Am. chem. Soc.* **102**, 7301–7302 (1980).
13. Peres, A. *J. Am. chem. Soc.* **102**, 7389–7390 (1980).
14. Kondepudi, D. K. & Nelson, G. W. *Physica* **125A**, 465–496 (1984).
15. Mason, S. F. & Tranter, G. E. *JCS chem. Commun.*, 117–119 (1983); *Molec. Phys.* **53**, 1091–1111 (1984).
16. Kondepudi, D. K. & Nelson, G. W. *Phys. Rev. Lett.* **50**, 1023–1026 (1983).
17. Kondepudi, D. K. & Prigogine, I. *Physica* **107A**, 1 (1981).
18. Sattinger, D. H. *Lecture Notes in Mathematics* No. 762 (Springer, Berlin, 1979).
19. Mangel, M. *J. chem. Phys.* **69**, 3697–3708 (1978).
20. Keizer, J. *J. chem. Phys.* **69**, 2609–2620 (1978).
21. Kondepudi, D. K. in *Fluctuations and Sensitivity in Nonequilibrium Systems* (eds Horsthemke, W. & Kondepudi, D. K.) 204–213 (Springer, Berlin 1984).
22. Kondepudi, D. K. & Nelson, G. W. *Phys. Lett.* **106A**, 203–206 (1984).
23. Mangel, M. *Phys. Rev.* **A24**, 3226–3238 (1981).
24. Morozov, L. L., Kuz'min, V. V. & Gol'danskii, V. I. *Origins of Life* **13**, 69–101 (1983).
25. Morozov, L. L., Kuz'min, V. V. & Gol'danskii, V. I. *Pis'ma Zh. exsp. teor. Fiz.* **39**, 344–345 (1984); *JETP Lett.* **39**, 414–416 (1984).
26. Nicolis, G. & Prigogine, I. *Self Organization in Nonequilibirum Systems* (Wiley, New York, 1977).
27. Nicolis, G. & Malek-Mansour, M. *Phys. Rev.* **A29**, 2845–2853 (1984).
28. Nitzan, A., Ortoleva, P., Deutch, J. & Ross, J. *J. chem. Phys.* **61**, 1056–1074 (1974).
29. Van Kampen, N. G. *Stochastic Processes in Physics and Chemistry* (North-Holland, Amsterdam, 1981).
30. Uhlenbeck, G. E. & Ornstein, L. S. *Phys. Rev.* **36**, 824–841 (1930).
31. Kuhn, W. & Braun, F. *Naturwissenchaften* **17**, 227–228 (1929).
32. Kagan, H. B. *et al Tetrahedron Lett.* **27**, 2479–2482 (1971).
33. Kagan, H. B., Balavoine, G. & Moradpour, A. *J. molec. Evol.* **4**, 41–48 (1974).
34. Flores, J. J., Bonner, W. A. & Massey, G. A. *J. Am. chem. Soc.* **99**, 3622–3624 (1977).
35. Angel, J. R. P., Illing, R. & Martin, P. G. *Nature* **238**, 389–390 (1972).

Presynaptic neurones may contribute a unique glycoprotein to the extracellular matrix at the synapse

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As the extracellular matrix at the original site of a neuromuscular junction seems to play a major part in the specificity of synaptic regeneration[1–5], considerable attention has been paid to unique molecules localized to this region[6–11]. Here we describe an extracellular matrix glycoprotein of the elasmobranch electric organ that is localized near the nerve endings. By immunological criteria, it is synthesized in the cell bodies, transported down the axons and is related to a glycoprotein in the synaptic vesicles of the neurones that innervate the electric organ. It is apparently specific for these neurones, as it cannot be detected elsewhere in the nervous system of the fish. Therefore, neurones seem to contribute unique extracellular matrix glycoproteins to the synaptic region. Synaptic vesicles could be involved in transporting these glycoproteins to or from the nerve terminal surface.

Cholinergic synaptic vesicles have been purified to near homogeneity from the electric organs of elasmobranchs[12,13] and have been used to generate nerve-terminal-specific antiserum[14,15]. Using synaptic vesicles purified from the electric organ of *Discopyge ommata* as antigen, we have generated monoclonal antibodies to unique vesicle components copurifying with synaptic vesicle contents during size fractionation. The vesicle-specific monoclonal antibodies define at least four different antigenic determinants (SV1–SV4). The SV1 region is part of the same proteoglycan-like molecule that binds monoclonal antibody tor70 (ref. 16). The other three sites have not yet been described. SV2 is fully accessible to antibody in intact vesicles, whereas the rest become accessible only after sonication or detergent treatment, and so are presumably exposed on the inside of the vesicle[17].

The relative concentration of each antigenic determinant was measured by quantitative immunoblotting of synaptic vesicles (Table 1). All four antigens are present in electric organ synaptosomes at about one-tenth of the concentration in vesicles, which is as expected because acetylcholine and ATP in the vesicles have ~10 times higher concentrations per mg protein[12] than in synaptosomes[18]. When the relative concentrations of all four determinants were measured on homogenates of electric organ (Table 1), SV2 and SV3 had low specific activities, as expected from earlier calculations[12]. The specific activity in the electric organ of the SV4 antigenic sites, however, was ~10 times higher than expected, suggesting that ~90% of it is not in synaptic vesicles. The SV4 site, unlike the other three, is found in high concentrations in an extracellular matrix fraction of the electric organ, where the specific activity is higher than in synaptic vesicles (Table 1). As 77% of the total SV4 (but only 2.1% of the total protein) is recovered in the extracellular matrix, most of the SV4 antigen in electric organ may not be in synaptic vesicles, but in the matrix.

The molecules carrying SV4 and SV1 are highly restricted in their location, whereas the SV2 and SV3 antigens occur in all nerve terminals of the electric fish. In homogenates of brain and spinal chord SV4 and SV1 were less than 2% of the value predicted from the amount of SV2 and equal to background levels measured using liver homogenates. By immunofluorescence the cell bodies of the electromotor nucleus contain large amounts of the SV2, SV1 and SV4 sites, whereas synapses in other brain regions have SV2 but no detectable SV4 or SV1 (K. Buckley, unpublished observations), hence the SV4 and SV1 antigens are restricted to electromotor nucleus neurones.

The molecule in synaptic vesicles carrying the SV4 site has a heterogeneous mobility and its antigenicity is destroyed by exposure to conditions[19] that remove carbohydrate side chains from proteins (Fig. 1). The molecule thus has the properties of a glycosylated protein whose antigenicity is associated with oligosaccharides. The SV4 antigen from the extracellular matrix fraction shows the same heterogeneous electrophoretic mobility, and the same susceptibility to mild proteolysis and sensitivity

Table 1 Relative amounts of unique antigenic determinants

	SV4	SV1	SV2	SV3
	(c.p.m. per μg protein) $\times 10^{-2}$			
Synaptic vesicles	111 ± 4	366 ± 12	170 ± 2	87 ± 2
Synaptosomes	17.4 ± 0.4	35 ± 1	14.8 ± 0.5	6.7 ± 0.5
Extracellular matrix	158 ± 3	18.4 ± 0.7	2.1 ± 0.9	1.2 ± 0.8
Electric organ homogenate	4.4 ± 0.4	3.8 ± 0.5	0.54 ± 0.1	0.32 ± 0.08

The concentration of antigenic sites was determined by an indirect solid-phase radioimmunoassay[16]. Samples were solubilized in 0.2 M NaCl, 10 mM HEPES, 1% SDS pH 7.0 and spotted on nitrocellulose filters. After saturating nonspecific sites with carrier protein (5% bovine serum albumin) monoclonal antibody was added in excess (1/100 dilution of hybridoma supernatants). Bound antibody was detected with affinity-purified iodinated goat anti-mouse IgG. The region where binding was linear with protein concentration was used to calculate antigen concentration by linear regression, ±s.e.m. Although the data from only one experiment are given, the relative ratios of antigen concentration have been confirmed at least four times. The extracellular matrix fraction was purified from the electric organ of *D. ommata* by the procedure of McMahan and his colleagues[11], except that detergent extraction was carried out at *p*H 10.5 to remove adventitiously adsorbed protein.

to deglycosylation as its vesicle counterpart, although the matrix-derived material seems to have an even slower electrophoretic mobility on polyacrylamide gels. Thus, the vesicle and extracellular matrix forms of the antigen are similar, but not identical.

Molecules bearing the SV4 antigen must form a tight association in the extracellular matrix fraction, as <10% is released by detergent extraction during matrix isolation. In contrast, >82% of the synaptic vesicle form is solubilized under identical conditions. The SV4 antigen can be solubilized completely from the extracellular matrix by high salt extraction (4M guanidinium chloride). Treatment of sonicated synaptic vesicles with either high salt or *p*H 11 buffer in the absence of detergents solubilizes <2% of the SV4 antigenic site, suggesting that it is part of an integral membrane protein.

Localization of the antigenic material in the synaptic extracellular matrix was confirmed by a combination of immunoelectron microscopy and biochemical data. Buckley *et al.*[20] have described an antibody to synaptic vesicles (tor70) which also binds to the outside of nerve terminals in the region where they face the electrocytes. SV1, SV3 and SV4 sites are also restricted to this extracellular domain (K. Buckley unpublished observations). To examine extracellular binding sites, we gently sheared electric organ to give large sheets of membrane covered with intact terminals[21,22].

To demonstrate that the SV4 antigenic sites in the extracellular matrix fraction correspond to those seen in the synaptic cleft in immunoelectron microscopy studies, antibodies were bound to the intact nerve terminals as before, except that ^{125}I-labelled goat anti-mouse second antibodies were used instead of fluorescent or peroxidase-labelled ones. With antibodies to the intravesicular antigens (SV1, SV3, SV4), the sheets became heavily labelled. When such sheets, labelled indirectly with antibodies to the SV4 antigen, were subjected to the standard fractionation procedure, 75% of the radioactivity occurred in the extracellular matrix fraction. In contrast, 3% of SV3 and 11% of SV1 were recovered in that fraction. (In this experiment the extracting buffer was at *p*H 7.5, not 10.5, to avoid dissociating antigen/antibody complexes.)

The SV4 antigenic site seems to be generated in the neuronal cell bodies; all four antigenic determinants could be measured in the axons and in the electromotor nucleus. To investigate whether these antigenic sites are generated in the periphery, the axons from this nucleus to the electric organ were cut. Three weeks later, the relative concentrations of the SV4 antigenic site in the electromotor nucleus had increased slightly (Table 2), suggesting that it is not derived by retrograde transport from the nerve terminals. Although SV2 and SV3 increased to the same extent, SV1 fell to 17% of its original concentration. To



Fig. 1 SV4 antigen exists on a glycoprotein. Synaptic vesicle proteins were deglycosylated by an acid hydrolysis procedure that specifically cleaves polysaccharide chains from glycoproteins[19]. Proteolytic digestion was performed for 5 min at 4 °C in the presence of 0.5% Nonidet P-40 with a twofold excess of trypsin over vesicle protein and was interrupted by boiling the sample in final sample buffer. Equivalent amounts of synaptic vesicle protein were run on an 8 M urea/8% SDS polyacrylamide gel and then transferred to nitrocellulose[27]. The nitrocellulose filters were then exposed to monoclonal antibody (1/100 dilution) and subsequently to ^{125}I-goat anti-mouse antiserum using standard washing procedures. Filters were then dried and exposed to Kodak X film with an intensifying screen for 15 h at −80 °C. Lane 1, untreated synaptic vesicles (2 μg protein); lane 2, deglycosylated synaptic vesicles (5 μg protein); lane 3, trypsin-digested synaptic vesicle proteins (2 μg vesicle protein, 4 μg trypsin). S, start of stacking gel; R, start of running gel; T, trypsin present in sufficiently high quantities to bind goat anti-mouse antibodies nonspecifically. The deglycosylation procedure[19] increased the electrophoretic mobility of a glycoprotein standard ovomucoid and did not destroy the antigenicity of the SV2 antigen but increased its relative molecular mass (data not shown).

show that the antigenic sites generated in the electromotor nucleus are transported to the nerve terminals, the nerves from the electromotor nucleus to the electric organ were crushed and the accumulation of antigens at the crush measured. SV1 accumulated only distally, implying that the SV1 antigenic site is probably generated at the nerve terminal (Table 2). SV4, on the other hand, increased three- to fourfold on the proximal side of the crush, implying anterograde transport, and also increased on the distal side, indicating retrograde transport.

As anterograde transport has been associated only with movement of newly synthesized material from the perikaryon[23], we propose that, unlike SV1, the SV4 antigen is made in the cell body. Direct support for a presynaptic origin of the SV4 antigen was obtained by injecting ^{35}S-methionine into the right lobe of the electromotor nucleus. Although the total concentration of label in the ipsi- and contralateral electric organs was indistinguishable (presumably because of systemic leakage of ^{35}S-methionine), immunoadsorption with anti-SV4 specifically precipitated 10–20% of the labelled protein from an extracellular matrix fraction of the right (injected) electric organ. In contrast, only 0–5% was precipitated from the contralateral extracellular matrix. Immunoprecipitation with a monoclonal antibody directed against a non-neural antigen showed no difference between the right and left sides. We therefore conclude that the SV4 antigen is synthesized in the electromotor nucleus and transported to the electric organ, and that the material that accumulated distal to a crush represents antigen that is recycling to the cell body from the nerve terminal. Analogous retrograde transport of vesicle proteins has been observed in vertebrate neurones[24–26] and has been interpreted as the reversal of anterograde flow at the nerve terminal[23].

Table 2 Demonstration that SV4 antigen is made in the cell body

Antigenic site/fraction	SV4	SV1	SV2	SV3
		(c.p.m. per μg protein) × 10^{-2}		
Electromotor nucleus	1.8 ± 0.1	0.83 ± 0.081	2.01 ± 0.2	0.6 ± 0.1
Electromotor nucleus after denervation	2.8 ± 0.2	0.14 ± 0.01	3.0 ± 0.3	1.00 ± 0.2
Nerve	0.33 ± 0.03	0.067 ± 0.009	0.026 ± 0.008	0.013 ± 0.009
Nerve proximal to crush	1.5 ± 0.2	0.09 ± 0.02	0.10 ± 0.01	0.05 ± 0.01
Nerve distal to crush	1.87 ± 0.06	0.52 ± 0.10	0.15 ± 0.02	0.06 ± 0.01

Conditions for operating on electric fish and denervating the electric organ have been described previously[22]. All the nerves to one of the two electric organs were cut and the animal was killed 3 weeks after surgery. The experimental and the control electromotor nuclei were removed separately. In axonal transport experiments, sections of nerves to the electric organ were crushed just proximal to their entry into the electric organ. Three days later the fish were killed and 2-4-mm segments proximal and distal to the crush removed. Samples were prepared by homogenization of tissue in 1% SDS buffer as before, and the antigen concentration measured by dot blotting. Specificity of antibody binding was verified by comparison with synaptic vesicle samples on Western blots.

Two more antigenic determinants shared by the synaptic vesicles and the extracellular matrix have been identified by raising monoclonal antibodies to electric organ extracellular matrix. Twenty-five of these bind to innervated sheets of electric organ membrane in a pattern indistinguishable from that obtained by Buckely *et al.*[20] with specific anti-synaptic vesicle antiserum. Two of these also bind to synaptic vesicle components that co-purify with vesicle contents on permeation chromatography. Both also block binding of ^{125}I-labelled antibody to the SV4 antigenic site on synaptic vesicles and on extracellular matrix, and have Western blot patterns on synaptic vesicle proteins similar to those in Fig. 1. Hence, all three antigenic sites are probably on the same molecule and physically close to each other but are not identical to SV4. The extracellular matrix (ECM)1 antigenic site closely resembled SV4 except that the ratio of extracellular matrix to vesicle binding sites differed by a factor of 2.5. Nerve crush experiments showed that, like SV1, ECM2 was only transported retrogradely and so is also probably generated at the nerve terminal. ECM1 and ECM2 are found only in the cell bodies and at the nerve terminals of the neurones innervating the electric organ. Only one synaptic vesicle component has been found in the extracellular matrix by immunological criteria; the extracellular matrix component is the only one found in vesicles. We conclude that three monoclonal antibodies to different but spatially contiguous antigenic sites bind to a glycoprotein of the synaptic extracellular matrix. The glycoprotein originates in the presynaptic neurone and is modified at the nerve terminal region.

Although the synaptic cleft region of the neuromuscular junction contains unique antibody and lectin-binding sites[6-8], the extracellular matrix glycoprotein recognized by these antibodies may be the first shown to be transported down the axon to the nerve terminals. Using the nerve cell to synthesize extracellular matrix precursors is an attractive solution to the problem of how to create synapse-specific domains in the extracellular matrix.

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1. Marshall, L. M., Sanes, J. R. & McMahan, U. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3073-3077 (1977).
2. Sanes, J. R., Marshall, L. M. & McMahan, U. J. *J. Cell Biol.* **83**, 357-370 (1978).
3. Burden, S. J., Sargent, P. B. & McMahan, U. J. *J. Cell Biol.* **82**, 412-425 (1979).
4. Sanes, J. R. *A. Rev. Physiol.* **45**, 581-601 (1983).
5. McMahan, U. J., Slater, C. R. & Marshall, R. M. *J. Cell Biol.* **98**, 1453-1473 (1984).
6. Sanes, J. R. & Hall, Z. W. *J. Cell Biol.* **83**, 357-370 (1979).
7. Burden, S. J. in *Monoclonal Antibodies to Neural Antigens* (eds McKay, R., Raff, M. C. & Reichardt, L. F.) 247-257 (Cold Spring Harbor Laboratory, New York, 1981).
8. Sanes, J. R. & Cheney, J. M. *Nature* **300**, 646-648 (1982).
9. Sanes, J. R. *J. Cell Biol.* **93**, 442-452 (1982).
10. Anderson, M. J. & Fambrough, D. M. *J. Cell Biol.* **97**, 1396-1411 (1983).
11. Godfrey, E. W., Nitkin, R. M., Wallace, B. G., Rubin, L. L. & McMahan, U. J. *J. Cell Biol.* **99**, 615-627 (1984).
12. Carlson, S. S., Wagner, J. A. & Kelly, R. B. *Biochemistry* **17**, 1188-1199 (1978).
13. Tashiro, T. & Stadler, H. *Eur. J. Biochem.* **90**, 479-487 (1978).
14. Sanes, J. R., Carlson, S. S., von Wedel, R. J. & Kelly, R. B. *Nature* **280**, 403-404 (1979).
15. Carlson, S. S. & Kelly, R. B. *J. Cell Biol.* **87**, 98-103 (1980).
16. Carlson, S. S. & Kelly, R. B. *J. biol. Chem.* **258**, 11082-11091 (1983).
17. Buckley, K. M. & Kelly, R. B. *J. Cell Biol.* (submitted).
18. Morel, N., Israel, M., Manaranche, R. & Mastour-Frachon, P. *J. Cell Biol.* **75**, 43-55 (1975).
19. Edge, A. S. B., Faltyeuck, C. R., Hof, L., Reichert, L. E. Jr & Weber, P. *Analyt. Biochem.* **118**, 131-137 (1981).
20. Buckley, K. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 7342-7346 (1983).
21. Morel, N., Manaranche, R. & Israel, M. *J. Physiol., Paris* **78**, 433-442 (1982).
22. Hooper, J. E., Deutsch, J. W., Miljanich, G. P., Brasier, A. R. & Kelly, R. B. *J. Physiol., Paris* **78**, 443-453 (1982).
23. Grafstein, B. & Forman, D. S. *Physiol. Rev.* **60**, 1167-1283 (1980).
24. Holtzman, E. *Neuroscience* **2**, 327-355 (1977).
25. Brimijoin, S. & Helland, L. *Brain Res.* **102**, 217-228 (1976).
26. Broadwell, R. D. & Brightman, M. W. *J. comp. Neurol.* **185**, 31-74 (1979).
27. Burnette, W. N, *Analyt. Biochem.* **112**, 195-203 (1981).

Carbon-dioxide-induced exocytotic insertion of H^+ pumps in turtle-bladder luminal membrane: role of cell *p*H and calcium

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The contents of endocytic vesicles and other intracellular organelles (such as Golgi and microsomes) are acidified by an electrogenic proton-translocating ATPase that is remarkably similar to that found in urinary epithelia[1-10]. We recently found that the number of H^+ ATPases in the apical plasma membrane of these epithelia is regulated by exocytotic insertion of endocytic vesicles whose membranes contain this H^+ pump[2,10]. Carbon dioxide, a major stimulus for urinary acidification, causes rapid fusion of these vesicles with the luminal membrane, thereby inserting these pumps there and increasing the rate of net transepithelial H^+ secretion; CO_2 also inhibits endocytic retrieval of the pumps from the luminal membrane[11]. Such reciprocal regulation of endocytosis and exocytosis by a physiological modulator makes this system particularly attractive for studying the cellular events regulating membrane fusion. Here we present evidence that CO_2 induces exocytosis by a cascade of events, the first step of which is cytoplasmic acidification. Cell acidification then increases calcium activity, which causes the fusion event.

The epithelial layer of the turtle urinary bladder is comprised of two cell types, one of which, the mitochondrion-rich cell, is enriched in carbonic anhydrase and in vesicles containing proton pumps; hence, it is thought to be the cell responsible for transepithelial H^+ transport[2,12]. When these bladders are mounted in Ussing chambers, H^+ secretion can be measured as the short-circuit current obtained when sodium transport is inhibited by ouabain[12]. To investigate whether CO_2 causes exocytotic insertion of H^+ pumps by acidifying the cytoplasm, we added 5 mM butyrate or acetate to the luminal medium and found that they stimulated the H^+ current in these bladders and that their effect became greater as the *p*H of the medium was reduced. At *p*H 7, butyrate increased the H^+ current by 33%, at *p*H 6 the H^+ current increased by 116%, whereas at *p*H 5 it increased by 179% ($n = 7$ experiments). Quantitatively similar results were obtained with acetate. Because the *p*K of butyrate and acetate is ~4.8 and the undissociated acid is more permeant, these results suggest that the species of the molecule that is

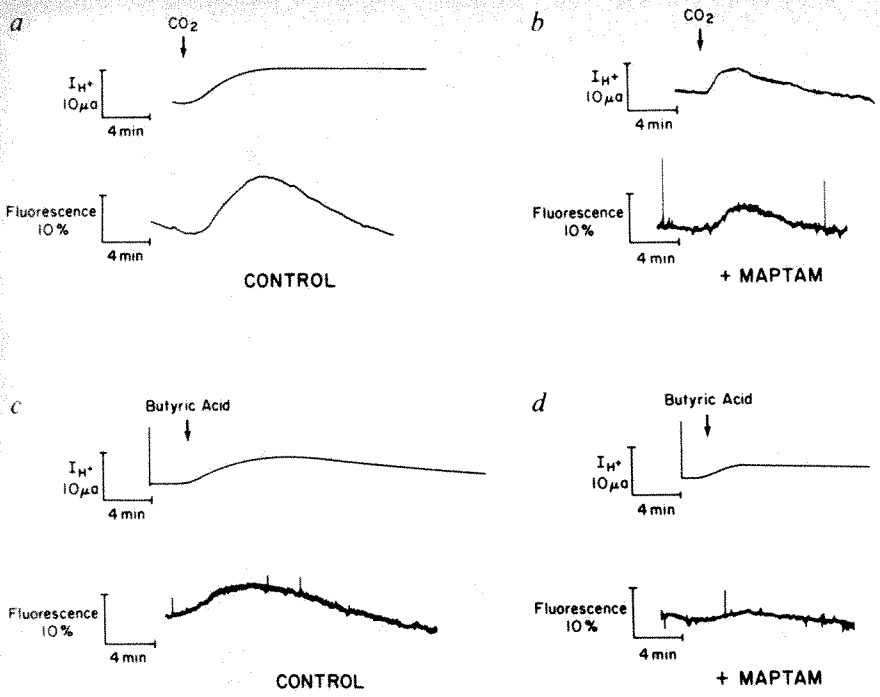


Fig. 1 Exocytosis of fluorescent dextran simultaneously with an increase in H^+ secretion in the turtle urinary bladder. The bladders were perfused on both sides by continuously circulating freshly aerated Ringer's solution with a peristaltic pump. The bladder was initially exposed on the luminal side for 1 h with a 5% CO_2 Ringer's (*p*H 7.5) with FITC dextran (relative molecular mass 70,000) dissolved at 100 mg ml^{-1} followed by 1 h of CO_2-free FITC dextran Ringer's. The luminal solution was then changed to CO_2-free Ringer's without FITC dextran *p*H 6.0 and was passed through a flow cell in a ratio fluorometer (Farrand Optical). After 1 h, both the H^+ current and the fluorescence fell to a low steady-state level. The luminal perfusate was then changed to one containing 5 mM butyric acid, *p*H 6.0 (*a*, *b*), causing a broad peak in the fluorescence tracing and an increase in the H^+ secretion. The solution was then changed to 5% CO_2 Ringer's *p*H 6.0 (*c*, *d*) and a similar rise in H^+ secretion and release of FITC dextran was found. *b*, *d* Show the effect of pretreatment of the luminal side of the bladders with 0.1 mM MAPTAM (Calbiochem) for 1 h.

Table 1 Effect of colchicine (*a*) and MAPTAM (*b*) on H^+ current in paired turtle hemibladders

a	Control	Colchicine-treated
Butyrate ($n=7$)	24±6	13±4*
Acetate ($n=7$)	10±3	4±4*
b	Control	MAPTAM-treated
CO_2 ($n=5$)	11.8±4.6	1.7±5*
Butyrate ($n=8$)	11.2±5	2.9±1.7*
Acetate ($n=3$)	3.0±0.8	0.8±0.6*

Paired hemibladders from freshwater turtles were mounted in Ussing chambers, bathed with CO_2-free Ringer's solution and continuously short-circuited using an automatic voltage clamp. The media were circulated by a gas-lift device using CO_2-free air. Ouabain (0.5 mM) was added to the serosal side in all experiments. The *p*H of the luminal solution was 6.0. The composition of the CO_2-free Ringer's solution was 85 mM NaCl, 3.5 mM KCl, 0.3 mM NaH_2PO_4, 1.65 mM Na_2HPO, 0.5 mM $MgSO_4$, 1 mM $CaCl_2$. *a*, Colchicine (0.1 mM) was added to one member of a pair and 2 h later 5 mM Na-butyrate or Na-acetate was added to the luminal side. The peak increase in the H^+ current is given. Current is given as μA per 8 cm^2 ± s.d. *b*, MAPTAM (50 μM dissolved in dimethyl sulphoxide) was added to one member of a pair whereas the other received diluent only. Two hours later, CO_2 was added isohydrically to both media. In other experiments, butyrate or acetate (5 mM each) was added to the luminal side only. In these experiments, the addition of CO_2 or the weak acids frequently resulted in rapid inhibition of the H^+ current.

*$P<0.05$ compared with the control value.

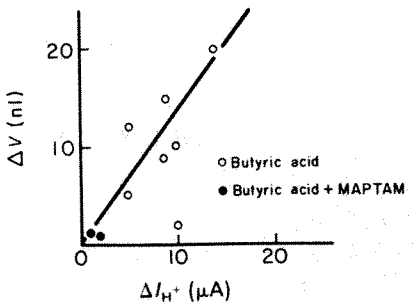


Fig. 2 Correlation of the volume of fluorescent dextran released with the rise in H^+ secretion. Known volumes of the fluorescent dextran loading solution were injected into the perfusion stream. The changes in the fluorescence signal were plotted against the volume injected to obtain a calibration curve from which the secreted volume was calculated.

increasing the transport rate is the undissociated weak acid, which would be expected to cause cytosolic acidification. The increase in H^+ secretion induced by butyrate or acetate was significantly reduced by pretreatment of the bladders with 0.1 mM colchicine (Table 1*a*), an agent that reduces the effect of CO_2 on exocytosis[2,10].

To provide direct evidence for exocytotic fusion induced by the weak acids, we measured their effect on the secretion of fluorescent dextran (fluorescein isothiocyanate (FITC) dextran) previously endocytosed by the bladder (see Fig. 1 legend for assay details). Addition of 5 mM butyrate stimulated secretion of FITC dextran simultaneously with an increase in H^+ secretion (Fig. 1*b*). As with CO_2 (Fig. 1*a*), the fusion event was transient whereas the increase in H^+ transport was constant, implying that the stimulation of H^+ transport was a result of the insertion of H^+ ATPases which continue to turn over and pump protons.

There was excellent correlation between the secreted volume, a function of the number of vesicles which fused, and the increase in H^+ current, which is some function of the number of pumps in the membrane (Fig. 2). These results provide strong evidence that butyrate, like CO_2, increases H^+ pumps in the membrane. Similar studies (not shown) showed that acetate also causes fusion of vesicles.

To measure the cell *p*H of individual mitochondrion-rich cells, we mounted turtle bladders on the stage of a fluorescence microscope and stained them with 6-carboxyfluorescein diacetate (6-CF). Because these cells are enriched in carbonic anhydrase, which is a potent esterase, they preferentially take up the dye and hence appear brighter than the other cell type (Fig. 3*e*). Measurement of the cell *p*H (using a microspectrofluorometric method described in Fig. 3 legend) showed that these cells are more alkaline by ~0.5 *p*H units than the principal cells, as might be expected from H^+-secreting cells. Addition of CO_2 or butyrate acidified the cytosol with a similar time course to that of the effect of these agents on fusion and H^+ transport (compare Figs 1 and 3). That these cells can maintain concentrations of H^+ and 6-CF different from their neighbours implies that they do not have patent gap junctions, a finding that suggests a separate developmental history of the two cell types.

Fusion of vesicles is frequently mediated by changes in cell calcium and recent studies suggest that CO_2 can alter cell calcium activity[13-16]. However, the effect of CO_2 varies in different cell types; in some cells there is an increase[14] while in others CO_2

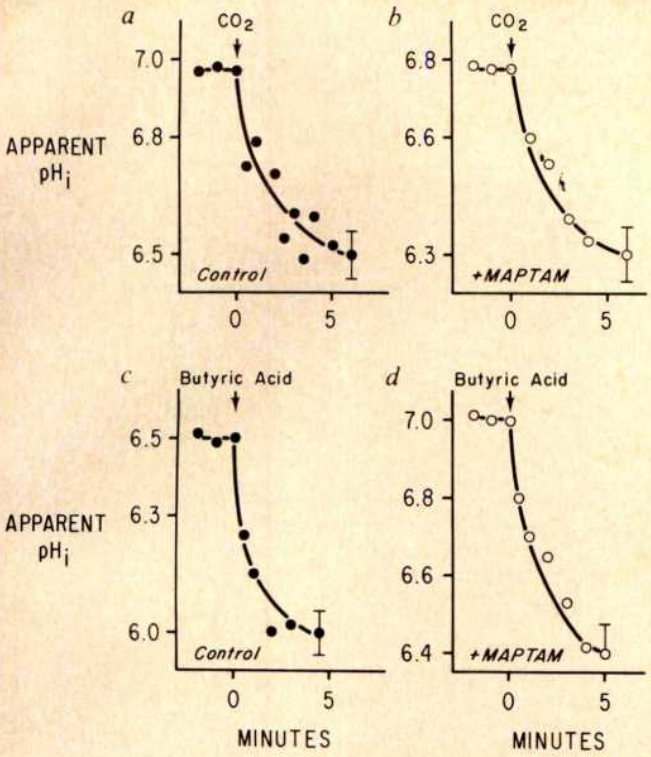




Fig. 3 *a–d*, Graphs showing the average drop in intracellular *p*H when the serosal perfusate was changed from CO_2-free Ringer's *p*H 7.5, to 5% CO_2 *p*H 7.5 (*a*, *b*) or 5 mM butyric acid Ringer's *p*H 7.5 (*c*, *d*). In *b* and *d*, the bladders were preincubated with 50 μM MAPTAM for 1 h. *e*, Turtle bladder epithelium stained with a *p*H-sensitive-fluorescent dye, 6-carboxyfluorescein diacetate (6-CF). Pieces (1 cm^2) of bladder were stretched across cut-off micro-centrifuge tubes. The luminal side of the bladders was stained for 4 min in 20 μM 6-CF in CO_2-free Ringer's *p*H 7.5. The cylinders were placed luminal side down on a glass coverslip on a Zeiss epifluorescence microscope equipped with a mercury lamp and a ×40 objective. The serosal side of the bladder was perfused continuously during microscopy. To measure the *p*H of the cytoplasm, we used a microfluorometer attached to the microscope (Farrand Optical)[2]. Single cells were centred in the aperture of the fluorometer and were excited alternately with 490-nm and 458-nm light using interference filters. The fluorescence emission intensity was measured at 530 nm with the microfluorometer; aperture, 22 μm. A calibration curve of the ratios of fluorescein intensities at the two exciting wavelengths against *p*H was obtained using 1 μM 6-CF dissolved in 0.14 M KCl, 1 mM $MgCl_2$ from *p*H 5.5 to 8.0. There were 7–12 experiments for each condition. The standard deviation, although shown only for the extremes, was the same for all points. The measurement of *p*H in a single cell by our method is subject to several problems relating largely to light scattering and background subtraction. Because each bladder has a different thickness, it is not possible to draw conclusions about the absolute value of the *p*H. However, changes in an individual cell were highly reproducible.

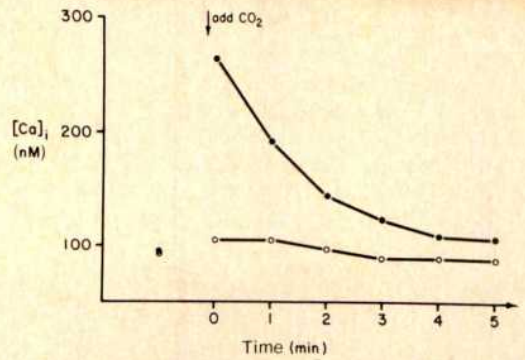


Fig. 4 Effect of CO_2 on intracellular calcium. ●, CO_2; ○, no CO_2. To measure intracellular calcium, turtle bladder epithelial cells were isolated by the method of Rossier *et al.*[23], except that no collagenase digestion was used. Cells were incubated for 1 h in 40 μM Quin 2 AM (Amersham) in CO_2-free Ringer's without calcium, *p*H 7.5. Cells were then washed and resuspended in CO_2-free Ringer's with 1 mM calcium. Fluorescence at excitation 340 nm with emission at 480 nm was measured with a Farrand spectrofluorometer using a 2.5-nm slit width. For each experiment, 1.5 ml of either CO_2-free Ringer's or 5% CO_2 Ringer's were added to 1.5-ml aliquots containing ~1.5×10^6 cells suspended in CO_2-free Ringer's, and the cuvette was sealed. Controls and experiments were always performed on the same cell suspension. Fluorescence was measured for 5 min, at which time calibration of measured Quin 2 fluorescence to concentration of calcium was performed by the method of Tsien[16,17] ($n = 7$, $P < 0.05$ for all points).

educes cell calcium[13,15,16] and in squid axon the effect appears o be biphasic[13]. To measure the changes in cell calcium, we ised the fluorescent calcium indicator Quin 2 in isolated uspended turtle bladder epithelial cells. Quin 2 is an EGTA nalogue which fluoresces when it binds calcium[17,18]; its ester s permeable and the active form is liberated in the cell by cytoplasmic esterases. In unstimulated cells, intracellular calcium is 95 ± 12 nM (±s.e.). Addition of CO_2 by diluting suspended cells with CO_2-saturated buffer causes an increase in cell calcium to a peak of 264 ± 92 nM ($n = 7$, $P < 0.05$), which declines with time towards control concentrations (Fig. 4).

Although these experiments were performed in suspensions containing both cell types, we believe that the signal observed mainly results from the mitochondrion-rich cells, as they were preferentially loaded with Quin 2 through the esterase activity of carbonic anhydrase. Pretreatment of this suspension with acetazolamide, an inhibitor of carbonic anhydrase, substantially prevents the loading of these cells by Quin 2 and 6-CF. Further, acetazolamide, which increases cell *p*H in these cells, reduces the resting calcium level[19]. However, studies in individually identified mitochondrion-rich cells are needed to provide conclusive evidence for the CO_2-induced increase.

To study whether the increased cell calcium is necessary for exocytotic insertion of H^+ pumps, we loaded the epithelium with the calcium buffer 1,2-*bis*-5-methyl-aminophenoxylethane-*N,N,N'*-tetraacetoxymethyl acetate (MAPTAM), another esterified but non-fluorescent EGTA derivative[17,18]. Addition of MAPTAM decreased the H^+ current slightly but considerably blunted the expected increase in H^+ secretion in response to CO_2 and butyrate (Table 1*b*). However, the response was quite variable; in some bladders there was still a small increase (Fig. 1*b*, *d*), while in others there was a rapid decline in the rate of H^+ secretion. On average (Table 1*b*), MAPTAM prevents the increase in H^+ secretion caused by CO_2 and butyrate. This effect is due to inhibition of fusion (Fig. 1*b*, *d*). MAPTAM has no effect on the cytoplasmic acidification caused by CO_2 or butyrate (Fig. 4*b*, *d*).

These results show that CO_2 causes exocytotic insertion of H^+ pumps initially by lowering cell *p*H, which in turn increases cytosolic calcium. The MAPTAM experiments suggest that the increased cell calcium is necessary for exocytosis. In preliminary experiments, we find that the cell *p*H, when lowered by CO_2, relaxes back to the original level in control cells but not in cells where the fusion event has been inhibited by removal of extracellular calcium. These results and the ubiquity of this H^+ ATPase in eukaryotic cells suggest that rapid insertion may be a new mechanism of cell *p*H regulation in addition to the well-studied one mediated by Na/H exchange[20]. In acid-transporting epithelia, this process would be modified only to the extent that

fusion occurs at one cell membrane. Further, recent studies on glucose-mediated insulin release[21] and the effects of CO_2 on vasopressin-induced water permeation[22] suggest that cell acidification may be a more widespread stimulus for exocytosis than previously thought.

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1. Tycko, B. & Maxfield, F. R. *Cell* **28**, 643-651 (1982).
2. Gluck, S., Cannon, C. & Al-Awqati, Q. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4327-4331 (1982).
3. Gluck, S., Kelly, S. & Al-Awqati, Q. *J. biol. Chem.* **257**, 9230-9233 (1982).
4. Forgac, M., Cantley, L., Wiedemann, B., Altstiel, L. & Branton, D. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1300-1303 (1983).
5. Stone, D. K., Xie, X.-S. & Racker, E. *J. biol. Chem.* **258**, 4059-5062 (1983).
6. Galloway, C. J., Dean, G. E., Marsh, M., Rudnick, G. & Mellman, I. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3334-3338 (1983).
7. Glickman, J., Croen, K., Kelly, S. & Al-Awqati, Q. *J. Cell Biol.* **97**, 1303-1308 (1983).
8. Rees-Jones, R. & Al-Awqati, Q. *Biochemistry* **23**, 2336-2340 (1984).
9. Gluck, S. & Al-Awqati, Q. *J. clin. Invest.* **73**, 1704-1710 (1984).
10. Schwartz, G. J. & Al-Awqati, Q. *J. clin. Invest.* (in the press).
11. Reeves, W., Gluck, S. & Al-Awqati, Q. *Kidney Int.* **23**, 232 (Abstr.) (1983).
12. Steinmetz, P. R. *Physiol. Rev.* **54**, 890-956 (1974).
13. Baker, P. F. & Honerjager, P. *Nature* **273**, 160 (1978).
14. Lea, T. J. & Ashley, C. C. *Nature* **175**, 236-238 (1978).
15. Lorenzen, M., Lee, C. O. & Windhager, E. E. *Fedn Proc.* **41**, 1350 (1982).
16. Alvarez-Leefmans, F. J., Rink, T. J. & Tsein, R. Y. *J. Physiol., Lond.* **315**, 531-548 (1981).
17. Tsien, R. Y. *Biochemistry* **19**, 2396-2403 (1980).
18. Tsien, R. Y., Pozzan, T. & Rink, T. J. *J. Cell Biol.* **94**, 325-334 (1982).
19. van Adelsberg, J. S. & Al-Awqati, Q. *Kidney Int.* **27**, 290 (abstr.) (1985).
20. Roos, A. & Boron, W. *Physiol. Rev.* **61**, 296-434 (1981).
21. Cook, D. L., Ikeuchi, M. & Fujimoto, W. Y. *Nature* **311**, 269-271 (1984).
22. Lorentzen, M., Taylor, A. & Windhager, E. E. *Am. J. Physiol.* **245**, F188-F197 (1983).
23. Rossier, M., Rossier, B. C., Pfeiffer, J. & Kraehenbuhl, J. P. *J. Membrane Biol.* **48**, 141-166 (1979).

The synthesis and *in vivo* assembly of functional antibodies in yeast

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The yeast *Saccharomyces cerevisiae* can synthesize, process and secrete higher eukaryotic proteins[1-5]. We have investigated the expression of immunoglobulin chains in yeast and demonstrate here (1) the synthesis, processing and secretion of light and heavy chains, (2) the glycosylation of heavy chain, (3) the intracellular localization of these foreign proteins by immunofluorescence, and (4) the detection of functional antibodies in cells co-expressing both chains. This may provide the basis of a microbial fermentation process for the production of monoclonal antibodies. The co-expression of light and heavy chains in *Escherichia coli* has been reported but functional antibodies were not assembled *in vivo*[6,7]. Furthermore, only low-level assembly of these chains was found *in vitro*.

The immunoglobulin λ and μ complementary DNAs used here were isolated from the mouse hybridomas S43 and B1-8, respectively[8,9]. Both hybridomas were raised against the hapten 4-hydroxy-3-nitrophenyl acetyl (NP). The λ and μ cDNAs were placed under the control of the yeast 3-phosphoglycerate kinase (PGK) promoter, on pMA91 bearing the *LEU2* selectable marker[10] (Fig. 1), to form pYλ for the expression of pre-λ, and pYμ for the expression of pre-μ. For co-expression of λ and μ on different plasmids in the same cell, another selectable marker was needed. pLG89 contains two selectable markers, *URA3* and *hph* (ref. 11), therefore the λ coding sequence and PGK promoter from pYλ were excised and inserted into pLG89, to produce pLGλ.

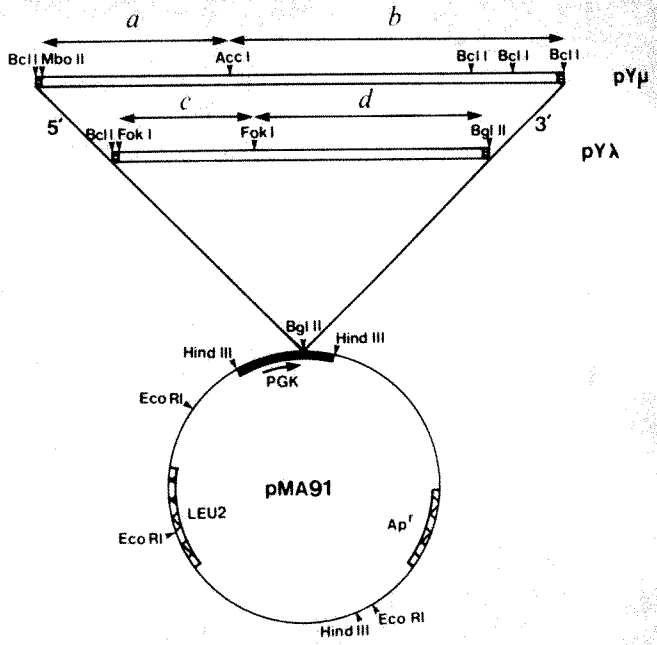


Fig. 1 Plasmids for the expression of immunoglobulin λ and μ chains in yeast. The figure shows the μ and λ inserts of pMA91 which were used to form pYμ and pYλ respectively. Solid bar, PGK sequences; the *LEU2* and Ap^r marker genes are cross-hatched. Sequences derived from synthetic oligodeoxyribonucleotides are indicated by horizontal bars. An arrow indicates the direction of PGK transcription.

Methods. All DNA manipulations were carried out as described elsewhere[22]. For construction of pYλ, the *Hin*dIII fragment of pATλ 1-15 (ref. 6) bearing the λ cDNA was isolated, and cut with *Fok*I; 307-base pair (bp) *Fok*I and 512-bp *Fok*I-*Hin*dIII fragments were isolated (fragments *c* and *d*, respectively). Both oligodeoxyribonucleotides[23]: R162 (5′GATCAATGGCCTG-GATT 3′) and R163 (5′GTGAAATCCAGGCCATT 3′) and pCT54 (ref. 24) cut with *Bcl*I and *Hin*dIII, to form pCTYλ. R162/R163 recreate the 5′ coding sequence, and place a *Bcl*I site immediately upstream of the initiation ATG. pCTYλ was digested with *Hin*dIII, blunted with S_1 nuclease and filled-in with T4 DNA polymerase (P-L Biochemicals); the product was ligated with *Bgl*II linkers (5′AGAGATCTCT 3′), then digested with *Bcl*I and *Bgl*II. The fragment bearing the λcDNA was isolated and ligated with *Bgl*II-cut pMA91, to form pYλ. The 5′ λ coding sequence of pYλ was isolated and shown to be correct by nucleotide sequencing. The λ coding sequence and PGK promoter were excised from pYλ on a *Hin*dIII fragment, and ligated with *Hin*dIII-cut pLG89 to form pLGλ. For construction of pYμ, the μ cDNA was excised from pCT54μ[25] on a *Hin*dIII fragment, blunted (as for pCTYλ) and ligated with linker R107 (5′TTTTGATCAAAA 3′) which contains an internal *Bcl*I site. The ligation product was digested with *Bcl*I and *Acc*I, and the fragment containing the 3′ end of μ coding sequence isolated (*b*). Only the *Bcl*I site created by R107 will cut, for internal sites were *dam*-methylated. pCT54μ was cut with *Mbo*II, ligated with R121 (5′GATCAATGGGATGGAGCTGT 3′) and R112 (5′CAGCTCCATCCCATT 3′) and digested with *Acc*I. The 276-bp *Bcl*I-*Acc*I fragment (fragment *a*) generated was isolated from a 5% polyacrylamide gel. pMA91 cut with *Bgl*II was ligated with both *a* and *b*, to form pYμ. The proximal end of the μ gene was isolated and shown to be correct by nucleotide sequencing[26,27]. Yeast transformations were carried out as described elsewhere[28]. pYλ and pYμ were individually transformed into MD46 (*a*/∝ *pep4.3*/*pep4.3 leu2*/*leu2 arg5.6*/+ +/*trp1* +/*rad52 ade1*/+ *his3*/+; Melanie Dobson, personal communication); pYμ and pLGλ were individually transformed or co-transformed into X4003-5B (*a leu2 ade1 his4 met2 ura3 trp5 gal1*; Yeast Genetic Stock Center, Berkeley, California). Cells containing pYλ or pYμ were selected for by growth in the absence of L-leucine, and pLGλ cells selected for by growth in the absence of uracil.

Cell extracts were subjected to Western blot analysis, using antisera to λ or μ proteins. MD46 cells containing pYλ were found to contain an immunoreactive protein (Fig. 2*a*, lane 3) that co-migrates with authentic B1-8 λ (Fig. 2*a*, lane 1) and with the mature λ protein synthesized by *E. coli* cells containing

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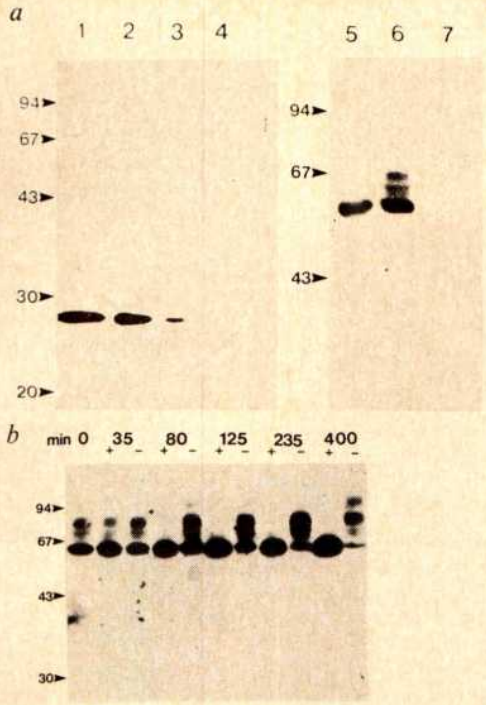


Fig. 2 Analyses of λ and μ proteins in yeast cell extracts. Cells were grown in YMM (2% (w/v) glucose, 0.67% (w/v) Difco yeast nitrogen base without amino acids) to $A_{660} = 1.0$, at 30 °C with shaking, then collected by centrifugation. Cell pellets were disrupted by vortexing with glass beads (40 mesh, BDH) in 50 mM Tris-HCl *p*H 7.6, 1 mM EDTA, and diluted in sample buffer to 1.5% (w/v) SDS, 2.5% (v/v) β-mercaptoethanol, 5% (v/v) glycerol. Samples were subjected to SDS-polyacrylamide gel electrophoresis[29] and transferred to 0.45-μm nitrocellulose and Western-blotted[30,31] with either rabbit anti-mouse λ antibody (Miles) or rabbit anti-mouse IgM (Bionetics), and challenged with affinity-purified iodinated protein A (2 μCi ml^{-1}; Amersham). Migration of yeast λ and μ was compared with that of B1-8 protein and of mature λ and μ synthesized in *E. coli*[6]. ***a***, Lanes 1–4, samples blotted with anti-λ antibody: lane 1, B1-8; lane 2, bacterial λ; lane 3, pYλ/MD46 cell extract; lane 4, MD46 cell extract. Lanes 5–7, samples blotted with anti-IgM: lane 5, bacterial μ; lane 6, pYμ/MD46 cell extract; lane 7, MD46 cell extract. Arrowheads indicate the positions of markers (Pharmacia): phosphorylase *b*, 94 K; bovine serum albumin, 67 K; ovalbumin, 43 K; carbonic anhydrase, 30 K; soybean trypsin inhibitor, 20.1 K. For secretion studies, cells were also grown in YPED (1% (w/v) Difco Bacto yeast extract, 2% (w/v) Difco Bacto peptone, 2% (w/v) glucose) and all cultures were buffered with potassium phosphate *p*H 7.0. In these conditions, we were able to detect the ADH activity in extracts from <0.005 A_{660} units of cells, which would represent lysis of <0.5% of a culture at $A_{660} = 1.0$. At such concentrations, ADH activity remained stable for up to 3 h at 30 °C. Medium supernatants were assayed for ADH by mixing in a cuvette 1 ml of supernatant with 0.3 ml of 500 mM potassium phosphate *p*H 7.0, 100 μl 30 mM NAD (reduced form; Sigma), 0.3 ml 30 mM acetaldehyde (BDH) and 1.3 ml double-distilled water. The decrease in A_{340} was measured with a Gilford spectrophotometer at 30 °C. ***b***, Demonstration of μ glycosylation in yeast. Duplicate 50-ml YMM cultures of MD46 containing pYμ were grown in 250-ml baffled flasks, with shaking at 30 °C to $A_{660} = 1.0$ then split into duplicate 20-ml cultures. To one culture, designated '+', we added tunicamycin[12,32] to a concentration of 15 μg ml^{-1}, in 30 μl of dimethyl sulphoxide (DMSO), and to the other culture, designated '−', we added 30 μl of DMSO alone. Cultures were left shaking at 30 °C, and samples taken at various times from both cultures. Cell extracts were prepared and Western-blotted with rabbit anti-mouse IgM and iodinated protein A. Alternating + and − tunicamycin samples are shown and the times of sampling given above the lanes in min. The 0-min sample was taken immediately before DMSO addition. Cells harbouring pYλ were treated similarly, and no difference was found between cultures with or without tunicamycin, when Western-blotted with anti-λ antiserum (data not shown). This result was expected as λ is a non-glycosylated protein in mammalian cells.

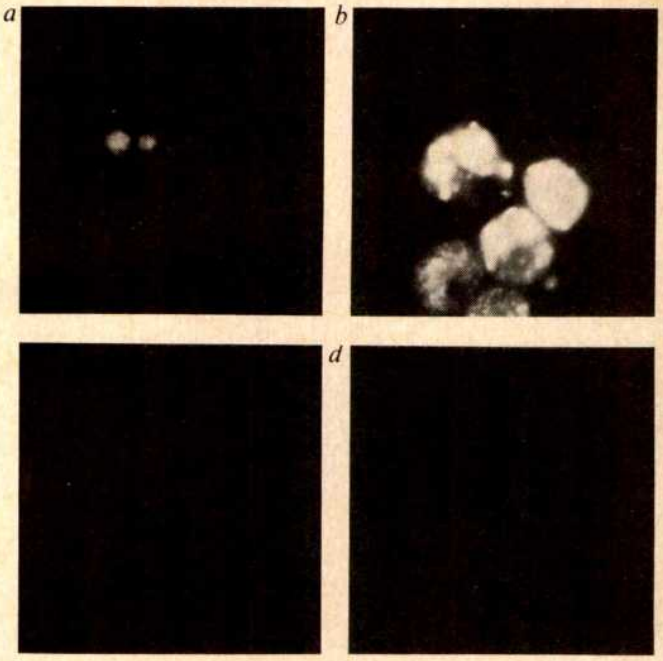


Fig. 3 Intracellular localization of λ and μ protein. The figure shows MD46 cells either untransformed (*c*, *d*), or transformed with pYμ (*a*) or pYMλ (*b*); *a* and *c* were incubated with fluorescein-conjugated anti-μ; *b* and *d* with rhodamine-conjugated anti-λ. ×700.

Methods. Cells were grown in minimal media and converted to spheroplasts with zymolyase 20,000 (50 μg per A_{660} unit in 0.5 ml; Miles), in 1.2 M sorbitol, 50 mM potassium phosphate *p*H 7.0, 15 mM β-mercaptoethanol, 10 mM EDTA, at 30 °C for 30 min. Spheroplasts were washed in 1.2 M sorbitol, and deposited on glass microscope slides using a cytocentrifuge, then fixed in 5% (v/v) acetic acid/95% (v/v) ethanol, at −20 °C overnight. Slides were rehydrated and washed thoroughly in phosphate-buffered saline (PBS), before being stained with 0.1 mg ml^{-1} of fluorescein isothiocyanate-labelled affinity-purified goat anti-mouse IgM or tetramethylrhodamine isothiocyanate-labelled affinity-purified goat anti-mouse λ (both from Southern Biotechnology Associates, Alabama). Slides were washed in PBS, and mounted in glycerol containing 1,4-diazabicylo(2.2.2)octane, before examination by ultraviolet microscopy.

a gene for mature λ (ref. 6) (Fig. 2*a*, lane 2). MD46 cells containing pYμ were found to contain three immunoreactive species (Fig. 2*a*, lane 6). The predominant yeast μ-immunoreactive band had a relative molecular mass (M_r) of ~63,500 (63.5 K) consistent with it being mature, non-glycosylated μ, and co-migrated with the mature μ synthesized by *E. coli* cells containing a gene for mature μ (ref. 6) (Fig. 2*a*, lane 5). In addition, two diffuse bands of greater relative molecular mass were found in MD46 cells containing pYμ (Fig. 2*a*, lane 6). The mature, glycosylated μ of B1-8 has a M_r ~70 K (not shown).

We conclude that the λ immunoreactive species is mature λ, and the ~63.5 K μ species is mature, non-glycosylated μ. On the basis of relative molecular mass, the signal sequences of both proteins have probably been cleaved. The higher-M_r μ bands were shown by two criteria to be glycosylated μ. First, the higher M_r μ was lost from cells treated with tunicamycin, an inhibitor of *N*-linked glycosylation[12]. In cultures without tunicamycin, the amount of higher-M_r μ increased during the time course examined, up to 235-min sample, after which the culture reached stationary phase, and the level of μ decreased (Fig. 2*b*). In cultures containing tunicamycin, the 63.5 K μ species accumulated with this species alone being found after 80 min of tunicamycin treatment. In addition, the higher-M_r form of μ was reduced in size to that of mature, non-glycosylated μ from *E. coli*, when treated with trifluoromethane sulphonic acid, which removes *N*-linked glycosyl groups with high efficiency[13] (data not shown). We conclude that a significant proportion of yeast μ is *N*-glycosylated. However, the yeast

and mammalian μ will probably differ in carbohydrate composition, especially if the yeast μ has outer-chain oligosaccharides[14,15].

Medium supernatants from cultures of MD46 cells transformed with either pYλ or pYμ were found to contain immunoreactive λ or μ protein, respectively. This was shown to be genuinely secreted material, rather than arising from cell lysis, by demonstrating the absence of a cytoplasmic enzyme, alcohol dehydrogenase (ADH), from the supernatants (see Fig. 2 legend). In yeast minimal medium (YMM), up to 10% of λ and 5% of μ in MD46 cultures at A_{660} ~1.0 was found in the medium supernatant, as detected by enzyme-linked immunosorbent assay (ELISA)[25]. In YPED medium (Fig. 2 legend) up to 40% of λ and 15% of μ was found in this fraction. Transformed X4003-5B cells generally yielded higher levels of secreted material. The immunoreactive λ material from the medium supernatants of cultures containing pYλ was shown, by Western blotting, to co-migrate with intracellular λ (data not shown). The secreted μ has not yet been examined.

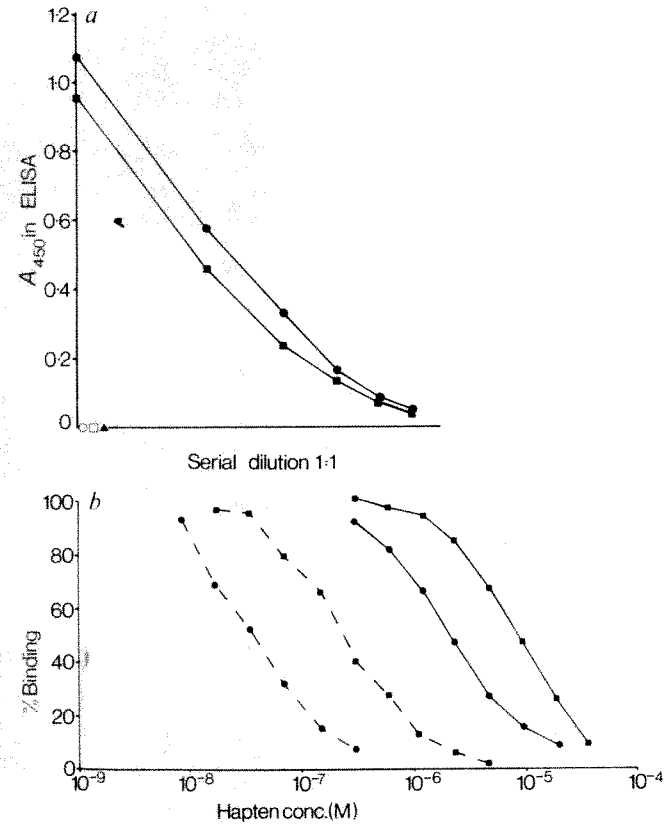


Fig. 4 Analysis of functional antibodies in yeast. Transformed X4003-5B cells were grown in 100-ml volumes of YMM, supplemented with 50 mg l^{-1} of L-tryptophan, L-histidine and L-methionine, and 30 mg l^{-1} of adenine sulphate in 500-ml flasks. Strains not containing pLGλ were supplemented with 30 mg l^{-1} uracil, and those not containing pYμ with 50 mg l^{-1} L-leucine. Cells were collected by centrifugation, then washed and resuspended in 25 mM Tris-HCl *p*H 8.0, 1 mM EDTA, 5% (v/v) glycerol, 0.1% (v/v) Nonidet-P40, 1 mM phenylmethylsulphonylfluoride (extraction buffer), before being lysed by glass bead disruption. 'Insoluble' material was pelleted in a microfuge for 12 min at 4 °C, and the 'soluble' supernatant fraction removed. The soluble fraction was analysed in a two-site sandwich ELISA (NIP assay) which detects μ-chain binding to haptenylated bovine serum albumin (NIP-caproate-BSA)[6]; this assay is sensitive to 60 pg of B1-8 IgM. *a*, Specific hapten binding was examined in the NIP assay of the soluble fraction of pLGλ + pYμ-transformed cells (●, ○), pYμ-transformed cells (▲) and B1-8 (■, □), in the absence (solid symbols) and presence (open symbols) of 30 μM free NIP-caproate. *b*, The heteroclitic nature of yeast antibody activity (●) in soluble fractions from pLGλ + pYμ-transformed cells and B1-8 (■) was examined by comparing binding in the presence of free NIP-caproate (- - -) and NP-caproate (——).

The intracellular locations of λ and μ proteins in yeast were examined by staining fixed yeast spheroplasts with fluorescein or rhodamine-conjugated antisera. We stained both MD46 and X4003-5B strains, harbouring pYμ, pYλ, pLGλ or pYMλ. pYMλ is a pMA91 derivative encoding the mature λ protein, that is, Met-mature λ, not including the signal sequence. Cells containing pYμ (Fig. 3*a*), pYMλ (Fig. 3*b*), pYλ or pLGλ showed a discrete immunofluorescence localized in bodies that appeared on the basis of their morphology to be vacuoles[16,17]. Cells containing pYMλ showed a much greater accumulation of stain than those containing pYλ; this may be explained by the observation that the intracellular concentration of mature λ from pYMλ is up to fourfold greater than that of pre-λ from pYλ, in MD46, by ELISA. Further experiments are required to identify unequivocally the sites of accumulation of immunoreactive λ and μ proteins. If these foreign proteins are being localized in the vacuole, they could be transported there by the secretory pathway[18,19], however, the detection of mature λ from pYMλ in the same structures calls this into question—the cell may be directing these proteins to the vacuole for degradation[20].

Soluble extracts of X4003-5B cells transformed with one or both of pYμ and pLGλ were prepared and analysed by an ELISA that detects binding of B1-8 to solid-phase hapten, in the presence and absence of competing free hapten[6] (Fig. 4*a*); no specific binding was detected for extracts of cells transformed with either pLGλ or pYμ. However, extracts of cells transformed with both plasmids, and expressing both light and heavy chains, showed a strong, specific signal similar to that of the hybridoma B1-8 protein. All detectable solid-phase hapten binding was lost in the presence of 30 μM free hapten. B1-8 is a heteroclitic antibody, and shows greater affinity for the related hapten 4-hydroxy-5-iodo-3-nitrophenyl acetyl (NIP), than for the hapten NP[21]. The yeast antibody activity also showed that higher free NP than free NIP concentrations were required to inhibit binding of antibody to solid-phase NIP (Fig. 4*b*). In addition, the activities of the yeast antibody and B1-8 showed similar specificity ratios (ratio of concentration of NP to NIP at 50% inhibition) of 37 and 47, respectively. The amount of μ protein in the soluble extract of cells containing pLGλ and pYμ was determined by ELISA, and using B1-8 as a standard, the specific activity of the yeast antibody was found to be ~0.5%. ELISA showed that at $A_{660} = 1.0$, there was ~500 ng of B1-8 λ equivalent, and 15 ng of B1-8 μ equivalent per ml. No significant amounts of NIP binding activity have been found in concentrated culture media (data not shown).

On the basis of the specific activity of the soluble-fraction yeast antibodies, the efficiency of assembly of functional antibodies is low, although the antibodies show both specific hapten binding and heterocliticity. It will be interesting to characterize further the yeast antibodies in the soluble fraction and to determine whether or not most of the immunoglobulin protein from the insoluble fraction (~75% of total) is also assembled into functional antibodies.

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1. Hitzeman, R. A. *et al. Science* **219**, 620-625 (1983).
2. Rothstein, S. J., Lazarus, C. M., Smith, W. E., Baulcombe, D. C. & Gatenby, A. A. *Natur* **308**, 662-665 (1984).
3. Valanzuela, P., Medina, A., Rutter, W. J., Ammerer, G. & Hall, B. D. *Nature* **298**, 347-35((1982).
4. Hitzeman, R. A. *et al. Nucleic Acids Res.* **11**, 2745-2763 (1983).
5. Brake, A. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4642-4646 (1984).
6. Boss, M. A., Kenten, J. H., Wood, C. R. & Emtage, J. S. *Nucleic Acids Res.* **12**, 3791-380((1984).
7. Cabilly, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 3273-3277 (1984).
8. Bothwell, A. L. M. *et al. Cell* **24**, 625-637 (1981).
9. Bothwell, A. L. M. *et al. Nature* **298**, 380-382 (1982).
10. Mellor, J. *et al. Gene* **24**, 1-14 (1983).
11. Gritz, L. & Davies, J. *Gene* **25**, 179-188 (1983).

12. Mahoney, W. C. & Duskin, D. *J. biol. Chem.* **254,** 6572-6576 (1979).
13. Edge, A. S. B., Faltynek, C. R., Hof, L., Reichert, L. E. & Weber, P. *Analyt. Biochem.* **118,** 131-137 (1981).
14. Chapman, A. & Kornfeld, R. *J. biol. Chem.* **254,** 816-823 (1979).
15. Ballou, C. E. in *The Molecular Biology of the Yeast* Saccharomyces, *Metabolism and Gene Expression* (eds Strathern, J. *et al.*) 335-360 (Cold Spring Harbor Laboratory, New York, 1982).
16. Schwencke, J. *Physiol. Vég.* **15,** 491 (1977).
17. Matile, P. *A. Rev. Pl. Physiol.* **29,** 193 (1978).
18. Schekman, R. & Novick, P. in *The Molecular Biology of the Yeast* Saccharomyces, *Metabolism and Gene Expression* (eds Strathern, J. *et al.*) 361-398 (Cold Spring Harbor Laboratory, New York, 1982).
19. Stevens, T., Esmon, B. & Schekman, R. *Cell* **30,** 439-448 (1982).
20. Lin, C.-J., Chopra, A. K., Strnadova, M. & Chaloupka, J. *FEMS Microbiol. Lett.* **21,** 313-317 (1984).
21. Imanishi, T. & Makela, O. *Eur. J. Immun.* **3,** 323-330 (1973).
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
23. Patel, T. P. *et al. Nucleic Acids Res.* **10,** 5605-5620 (1982).
24. Emtage, J. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 3671-3675 (1983).
25. Wood, C. R., Boss, M. A., Patel, T. P. & Emtage, J. S. *Nucleic Acids Res.* **12,** 3937-3950 (1984).
26. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463-5467 (1977).
27. Messing, J., Crea, R. & Seeburg, P. H. *Nucleic Acids Res.* **9,** 302-321 (1981).
28. Beggs, J. *Nature* **275,** 104-109 (1978).
29. Laemmli, U. K. *Nature* **227,** 680-685 (1970).
30. Burnette, W. N. *Analyt. Biochem.* **112,** 195-203 (1981).
31. Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4350-4354 (1979).
32. Onishi, H. R., Tkacz, J. S. & Lampen, J. O. *J. biol. Chem.* **254,** 11943-11952 (1979).

Functional modifications of cytotoxic T-lymphocyte T200 glycoprotein recognized by monoclonal antibodies

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Plasma membrane glycoproteins of cytotoxic T lymphocytes (CTLs) are involved in the binding to and subsequent destruction of appropriate target cells[1–3]. The electrophoretic profile of surface proteins of mature CTLs, particularly those of high relative molecular mass (M_r), is markedly different from that of naive peripheral T cells or non-cytolytic T cells[4–7], suggesting the possible involvement of these molecules in the activation of CTLs and/or in the lytic process itself. By generating monoclonal antibodies to cell-surface proteins of CTL clones, we have now detected CTL-specific modifications in one of these high-M_r membrane proteins, T200. Although forms of T200 are found on a wide variety of cell types, the neoantigenic determinants recognized by our antibodies are present exclusively on activated T cells and in high concentrations only on CTLs. Furthermore, the expression of the modifications recognized by our antibodies is influenced by soluble factors and also seems to have functional significance, as monoclonal antibodies specific for these novel epitopes block cytolytic activity.

Monoclonal antibodies with specificity for CTL surface recognition structures were produced by immunizing BALB.B mice repeatedly (intraperitoneally) with a C57BL/6-derived CTL clone, B3.3, which is specific for a BALB minor histocompatibility antigen in association with H-2K^b. Spleen cells of immune mice were fused to a myeloma partner, P3-X63 Ag8.653, and the resulting hybridoma supernatants were screened for the ability to block specific target lysis by B3.3. Figure 1*a* depicts the blocking activity of two of these monoclonal antibodies, CT1 and CT2, on clone B3.3 in conditions of saturating antibody. CT1 and CT2 (which are both IgM antibodies) efficiently block specific killing of BALB.B target cells by clone B3.3, CT2 being the more efficient of the two antibodies. In this regard, CT2 was as efficient as the anti-Lyt-2 monoclonal antibody, 53.6.72. Antibody I3/2.3, which is specific for the T200 molecule[8], did not significantly affect specific lysis by this CTL clone.

To determine whether CT1 and CT2 were able to block CTL-mediated killing in conditions not requiring antigen-specific recognition on the part of the CTLs, we tested their ability to inhibit lectin-dependent cell-mediated cytotoxicity (LDCC). Both antibodies were highly efficient as inhibitors of

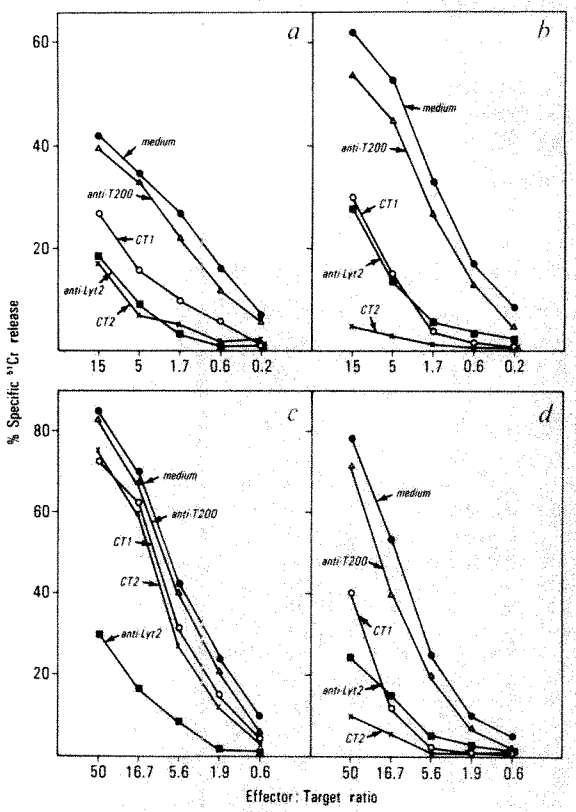


Fig. 1 Inhibition by CT antibodies of cytolysis mediated by cloned CTLs and MLC-derived CTLs. Serial dilutions of effector cells were incubated in 96-well round-bottomed microtitre plates for 30 min at 4 °C with saturating levels of CT1 (○), CT2 (×), I3/2.3 (△, anti-T200, kindly provided by Dr Ian Trowbridge, Salk Institute), 53.6.72 (■, anti-Lyt-2), or medium (●). Effector cells were: *a*, *b*, CTL clone B3.3 (a C57BL/6 clone derived by limiting dilution and specific for a BALB minor histocompatibility antigen in association with H-2K^b); *c*, C57BL/6 spleen cells stimulated for 5 days with irradiated DBA/2 spleen cells (5×10^6 cells of each per well in 24-well plates) in RPMI 1640 culture medium supplemented with 5% fetal calf serum; *d*, MLC cells derived as in *c* but restimulated (5×10^5 responders and 5×10^6 stimulators per well) every 7 days for 4 weeks in medium containing 5% rat Con A supernatant (RCS). ^{51}Cr-labelled target cells (1×10^4) were as follows: *a*, 3-day BALB.B Con A blasts; *b*, P815 H-2^d mastocytoma cells with the addition of 10 μg ml^{-1} PHA; *c*, *d*, P815 cells. After the addition of target cells, the plates were centrifuged for 3 min at 800 r.p.m. to initiate cell contact and incubated at 37 °C for 2 h. Per cent specific lysis was calculated as $100\times$[(c.p.m. released with effectors) − (c.p.m. released alone)]/[(c.p.m. released by detergent) − (c.p.m. released alone)]. Spontaneous release of P815 target cells was 5% and that of BALB.B Con A blasts 18%. No significant lysis was observed of C57BL/6 Con A blasts (*a*), of P815 cells without the addition of PHA (*b*), or of EL4 cells (*c*, *d*).

phytohaemagglutinin (PHA)-dependent killing of P815 tumour cells by clone B3.3 (Fig. 1*b*). CT1 was comparable with anti-Lyt-2 in blocking LDCC, while the anti-T200 antibody did not inhibit lysis (Fig. 1*b*). (Note that CT1 and CT2 do not bind to the target cells used in these assays; see below.) We also found that five out of five independently isolated CTL clones of various target specificities were blocked by both antibodies. Thus, it was apparent that CT1 and CT2 were not clone-specific or anti-idiotypic in their reactivity. More surprising results were obtained when we examined their effect on heterogenous populations of CTLs generated in mixed lymphocyte culture (MLC). When the effects of these antibodies on the lytic ability of a primary MLC were tested (Fig. 1*c*), only minimal inhibition of specific lysis was observed using CT1, CT2 or the control antibody I3/2.3 (anti-T200), while 53.6.72 (anti-Lyt-2) significantly inhibited lysis. However, quite surprisingly, in inhibition assays using as effectors CTLs from a long-term MLC, CT1 and CT2 produced a significant decrease in specific lysis (Fig. 1*d*). This MLC (and others similarly blocked by CT1 and CT2) were

Table 1 CT antigen expression in cytolytic and non-cytolytic T-cell populations and in naive cell populations

Cell source	Strains represented	Reactivity
Cytotoxic T-cell clones and long-term MLC cells (15)	C57BL/6, SJL, BALB.B, BALB/c, B10.D2, B10.BR	++++
Non-cytolytic T-cell clones (11)	C57BL/6, B10.D2, CAF_1, B10.A	+/−
Non-cytolytic T-cell hybrids (10)	B10.A, B10.D2	−, +/−
Primary MLC cells	C57BL/6, BALB.B	++
Thymocytes	C57BL/6, BALB.B	−
Lymph node T cells	C57BL/6, BALB.B	−
Bone marrow	C57BL/6	−
Spleen	C57BL/6	−

Reactivity of CT1 and CT2 was determined by fluorescence flow cytometry or fluorescence microscopy. CTL clones, long-term MLC cells and non-cytolytic T-cell clones were propagated by restimulation with the appropriate irradiated spleen cells in medium containing 5% supernatant derived from Con A-stimulated rat spleen cells (RCS) and 50 mM α-methyl mannoside. Non-cytolytic T-cell hybrids and primary MLC cells were grown in medium without RCS. MLCs were established by co-culturing 5×10^6 responder spleen cells per 2 ml culture with 5×10^6 irradiated (2,000 rad, ^{137}Cs source) stimulator spleen cells. Primary MLC cells were tested for CT reactivity 5 days after stimulation and long-term MLC cells were tested 4 days after the fifth *in vitro* restimulation. Viable cells were isolated by passage over Ficoll-Isopaque, resuspended in phosphate-buffered saline with 0.1% NaN_3 and 0.1% bovine serum albumin at a concentration of 1×10^7 ml^{-1}. Cells (1×10^6) were incubated with 100 µl of a 1:100 dilution of ascites fluid of antibodies CT1 or CT2 at 4 °C for 30 min. After washing, the cells were incubated for 30 min at 4 °C with a fluorescein-conjugated affinity-purified $F(ab')_2$ rabbit anti-mouse reagent (Cappel Laboratories). After washing, the cells were resuspended at 1×10^6 cells ml^{-1} for cytofluorimetric analysis or were placed directly on slides for fluorescence microscopic analysis. Numbers in parentheses indicate the number of individual clones tested. For comparison of relative fluorescence intensities, see Fig. 3.

cultured in the presence of supernatants containing interleukin-2 (IL-2), as were our CTL clones. Only weak blocking of long-term MLC cells was observed without the addition of exogenous IL-2 to the cultures. This dichotomy seems to result from differential expression of CT antigens on MLC cells cultured with or without lymphokine-rich supernatants (see below).

As these antibodies clearly were reacting with CTLs, we also investigated the presence of the antigens recognized on other cell populations. By screening a large panel of T-cell clones for reactivity with CT1 and CT2, we determined that only T cells of CTL lineage expressed significant levels of the antigen(s) recognized by the CT monoclonal antibodies (Table 1). Seventeen independent CTL clones expressed high levels of CT antigen whereas 11 non-cytolytic T-cell clones and 10 non-cytolytic T-cell hybrids either did not express the CT determinants or expressed them only weakly. Importantly, both CTL clones and non-cytolytic T-cell clones were cultured identically in the presence of antigen and IL-2-containing supernatants. Furthermore, the T-cell clones tested represent a wide variety of specificities and strains of origin, indicating that the genetic background of the T cells probably does not influence CT antigen expression. In fact, these antibodies are autoantibodies, as they were produced in BALB.B mice and react with BALB.B-derived CTL. Primary MLC cells express intermediate levels of CT antigen whereas thymocytes, lymph node T cells, spleen cells and bone marrow cells were unreactive with the CT antibodies. Several T-cell lymphoma lines tested also did not express the CT antigens[9].

The characteristics of CT1 and CT2 suggested the presence of a CTL-specific differentiation or activation antigen(s) involved in CTL-induced lysis. The biochemical characteristics of the antigen recognized by these antibodies were explored by radioiodination of the CTL clone B3.3 followed by immune precipitation (Fig. 2). Precipitation with CT1 followed by SDS-

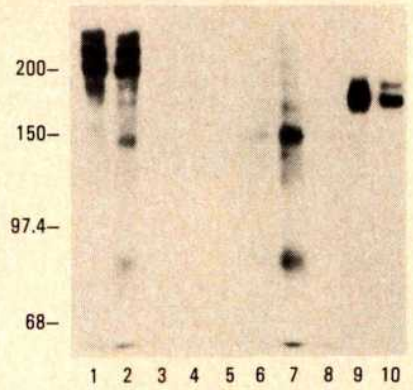


Fig. 2 Radioimmunoprecipitation by anti-T200 and CT1 antibodies. CTL clone B3.3, EL4 thymoma cells or the non-cytolytic M1s-reactive T-cell clone BB5 (kindly provided by Dr Andrew Glasebrook, Salk Institute) were labelled using $Na^{125}I$ (1.0 mCi per 1×10^7 cells) by the lactoperoxidase method[28]. After washing, the cells were resuspended in lysis buffer (phosphate-buffered saline, 1% Nonidet P-40 (NP40), 2 mM phenylmethylsulphonyl fluoride, 20 IU ml^{-1} aprotinin, 10 mg ml^{-1} bovine serum albumin) and incubated on ice for 30 min. The lysates were centrifuged at 13,000 *g* for 5 min and aliquots of the supernatant were incubated with ascites fluid containing CT1 or, in the case of I3/2.3, with the monoclonal antibody directly coupled to Sepharose 4B (a gift of Dr Ian Trowbridge). For precipitations using CT1, protein A-Sepharose 4B coated with rabbit anti-mouse immunoglobulin was used as a second-step reagent. After incubation at 4 °C, the immune complexes were washed extensively with buffer (20 mM Na-phosphate, 0.5 M NaCl, 0.5% deoxycholic acid, 0.5% NP40, 0.1% SDS, 2 mM EDTA) and subsequently analysed by SDS-polyacrylamide gel electrophoresis according to the procedure of Laemmli[29] but using as acrylamide stock a 30% w/v acrylamide, 0.17% bis-acrylamide solution to form 7.5% acrylamide gels. The gels were dried and subjected to autoradiography. Lanes 1–8, CTL clone B3.3. Lane 1, I3/2.3 (anti-T200); lane 2, CT1; lane 3, CT1 after preclearance with CT1; lane 4, I3/2.3 after preclearance with CT1; lane 5, I3/2.3 after preclearance with I3/2.3; lane 6, CT1 after preclearance with I3/2.3; lanes 7, 8, prolonged autoradiographic exposure of lanes 6, 5, respectively. Lanes 9, 10, I3/2.3 precipitates of labelled BB5 and EL4 cell lysates, respectively. M_r markers are shown$\times10^{-3}$.

polyacrylamide gel electrophoresis (PAGE) yielded a series of proteins of high M_r as well as two proteins of relatively lower M_r (Fig. 2, lane 2). The high M_r proteins recognized by CT1 appeared similar to the molecules precipitated from the same lysates by the anti-T200 antibody I3/2.3 (Fig. 2, lane 1). CT1 precipitated proteins with apparent M_rs of 24,000 (24K), 220K and 200K and two proteins with lower apparent M_rs of 140K and 85K. Minor proteins of 190K and 180K were also occasionally observed. Precipitation with anti-T200 antibody followed by PAGE showed nearly the same high-M_r protein profile as that precipitated by CT1, with a greater amount of the 190K protein being precipitated by anti-T200. Interestingly, the 140K and 85K proteins precipitated by CT1 were never observed in anti-T200 immmunoprecipitates. Although CT2 was very inefficient in immunoprecipitation experiments, Western blot analysis with both antibodies showed similar binding profiles (data not shown).

We next investigated whether the determinants recognized by CT1 and the anti-T200 antibody were present on the same molecules. Sequential immunoprecipitations were carried out using CT1 or anti-T200 to preclear lysates from surface -labelled cloned CTLs. Preclearing of the lysate from clone B3.3 with CT1 resulted in the complete removal of the antigens recognized by CT1 (Fig. 2, lane 3) and by anti-T200 (lane 4). Conversely, preclearing with anti-T200 followed by precipitation with anti-T200 or CT1 revealed that most of the high-M_r material reactive with CT1 or anti-T200 had been removed (Fig. 2, lanes 5, 6). However, the two lower-M_r proteins recognized by CT1 were not removed by preclearance with anti-T200 (Fig. 2, lanes 6, 7).

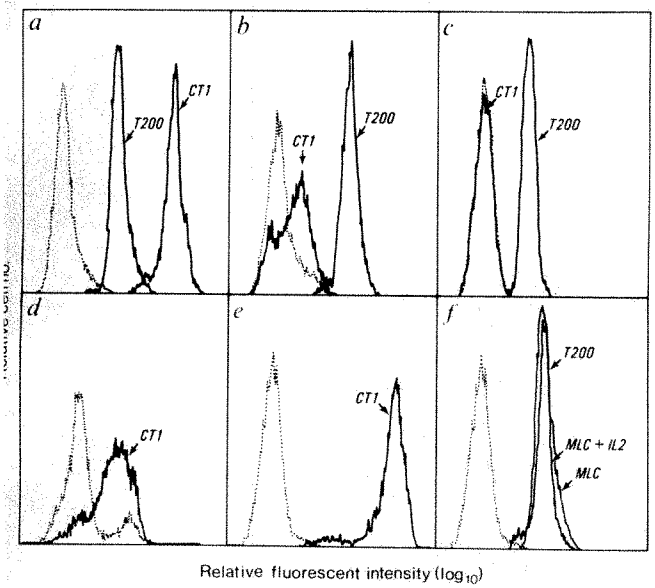


Fig. 3 The preferential reactivity of CT monoclonal antibodies with CTLs is not due to variations in cell-surface T200 concentration. *a*, CTL clone B3.3; *b*, T-helper clone D10.2; *c*, thymocytes; *d*, primary MLC; *e*, long-term MLC cultured with RCS; *f*, long-term MLC with or without the addition of RCS. Viable cells were stained as in Table 1 with CT1 or I3/2.3 (anti-T200) followed by reaction with an $F(ab')_2$ of a fluorescein-rabbit anti-mouse immunoglobulin (CT antibodies) or fluorescein rabbit anti-rat immunoglobulin (I3/2.3). Relative fluorescent intensities of individual cells were measured using the FACS-IV (Becton-Dickinson). Forward angle light scatter was used to exclude dead and aggregated cells. Dotted lines indicate the fluorescent profile of control antibody. Non-cytolytic T-cell clone D10.2 was provided by Dr Susan Webb.

For comparison of the T200 molecule present on CTLs with he T200 protein of other T-cell populations, the non-cytolytic T-cell clone BB5 and the EL4 thymoma were suface-labelled ınd the lysates immunoprecipitated with anti-T200 (Fig. 2, lanes), 10). PAGE analysis showed that two 175K and 190K proteins were precipitated from the surface of EL4 cells (a minor protein of 200K was also sometimes visible) and a heterogeneous protein(s) of 175–195K was precipitated from BB5. The T200 protein profile of EL4 was virtually identical with that of thymocytes ınd lymph node T cells. Immunoprecipitation with CT1 revealed hat a minor amount of T200 ($<1\%$ of total precipitable T200) was precipitated from BB5 cell lysates while no specific precipiation of proteins by CT1 from EL4 lysates was observed[9].

Because the CT determinants are expressed on T200 nolecules, their differential expression could result from disparıte T200 levels on different cell populations. To test this possibilty, we analysed T-cell populations by fluorescence flow ytometry after reactions with CT or anti-T200 antibodies folowed by reaction with a fluorescein-labelled secondary reagent Fig. 3). CTL clone B3.3 expressed high levels of CT deterninants (Fig. 3*a*), whereas an Mls-reactive non-cytolytic T-cell lone D10.2 (which produces IL-2 in response to antigen) xhibited very low reactivity with the CT antibody, ~25% of he cells being weakly CT^+ (Fig. 3*b*). However, the level of ell-surface T200 on these clones was nearly identical. Thynocytes were unreactive with CT antibodies but displayed a evel of T200 similar to that of the T-cell clones (Fig. 3*c*). Primary MLC cells (which were ~85% Thy 1^+ and ~65% Lyt-2^+) xpressed intermediate levels of CT antigen, with ~60% of ells being CT^+ (Fig. 3*d*). In contrast, virtually all long-term MLC cells cultured with exogenous IL-2 (>95% Thy 1^+, $T200^+$ ınd Lyt-2^+) expressed very high levels of CT antigen (Fig. 3*e*). The T200 concentration on long-term MLC cells cultured with ır without exogenous IL-2 remained constant (Fig. 3*f*), indicatng that different levels of CT antigen expression were not the esult of variations in T200 expression. The shift from low to ıigh CT antigen expression is accompanied by marked sequenial biochemical changes in the T200 molecule[9].

The T200 glycoprotein, which carries the Ly 5 determinants[10,11], has been implicated in several immunological mechanisms[12–14] and is a major cell-surface receptor for concanavalin A (Con A)[15,16]. The inhibitory activity of anti-Ly 5 sera or anti-T200 monoclonal antibodies on CTLs has remained a controversial topic[17–21]. (Note that the CT antibodies bind to long-term MLC cells and T-cell clones from both B6 (Ly 5.1) and SJL (Ly 5.2) mice, Table 1.) Our results indicate that the CT antibodies are able to inhibit CTLs expressing high levels of CT antigen but are unable to block CTLs exhibiting relatively low CT antigen levels (long-term MLC cells compared with primary MLC cells; Fig. 3*d*, *e*). However, primary MLC cells do express significant levels of CT antigen whereas naive peripheral T cells and clones of T-helper cell phenotype do not. The inability of CT antibodies to inhibit primary MLC CTLs does not exclude a role for CT determinants in the general mechanism of CTL-induced lysis, as the inhibition assay may be influenced by various parameters, including antigen expression (as shown), the relative affinities of monoclonal antibodies and CTLs, and CTL recycling.

Few antigens have been described that are specific for activated T cells, and no monoclonal antibodies specific for activation determinants of a T-cell subclass have been reported until now. By selectively labelling T-cell surface glycoproteins, Kimura and Wigzell[22] demonstrated the presence of a 145K protein expressed primarily on Lyt 1^-2^+T cells, but drew no conclusions as to the functional role of this molecule. Whether this protein is related to the 140K protein precipitated by CT1 is unknown. The monoclonal antibody HNK-1, which is reactive with carbohydrate, defines a differentiation antigen selectively expressed on human natural killer cells[23,24] and is therefore similar to the CT antibodies in defining a functional subset of lymphocytes. Indeed, our preliminary results suggest the CT antibodies may also be specific for cell-type-specific carbohydrate determinants. Interestingly, a variant of T200, termed T200A, selectively appears on most T cells after activation[25]. However, unlike the antigens described here, the T200A determinants are present on molecules distinct from the T200 glycoprotein and monoclonal antibodies specific for T200A do not inhibit CTL-mediated cytolysis[25]. Recent work has also indicated the presence on human T cells of high-M_r activation antigens distinct from the human T200 molecule[26,27]. Thus, activation of T cells results in the appearance of proteins or antigenic determinants not present on resting T cells which are apparently involved in their subsequent functions. We propose that the maturational modifications of the T200 glycoprotein detected by our antibodies are functionally related to the lytic capability of CTLs. These alterations may act to stabilize further the interaction of CTL and target cells or may represent modifications necessary for the triggering of the lytic mechanism in fully differentiated CTLs.

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1. Martz, E., Heagy, W. & Gromkowski, S. H. *Immun. Rev.* **72**, 73–96 (1983).
2. Meuer, S. C. *et al. J. exp. Med.* **157**, 705–719 (1983).
3. Sarmiento, M. *et al. Immun. Rev.* **68**, 135–169 (1982).
4. Dunlap, B. *et al. J. Immun.* **125**, 1829–1831 (1980).
5. Sarmiento, M., Glasebrook, A. L. & Fitch, F. W. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1111–1115 (1980).
6. Dunlap, B. *et al. Trans. Proc.* **13**, 1143–1146 (1981).
7. Tung, J. S., Scheid, M. P. & Falladino, M. A. *Immunogenetics* **17**, 649–654 (1983).
8. Trowbridge, I. S. *J. exp. Med.* **148**, 313–323 (1978).
9. Lefrancois, L. & Bevan, M. J. *J. Immun.* (in the press).
10. Omary, M. B., Trowbridge, I. S. & Scheid, M. P. *J. exp. Med.* **151**, 1311–1316 (1980).
11. Siadak, A. W. & Nowinski, R. C. *J. Immun.* **125**, 1400–1401 (1980).
12. Yakura, H., Shen, F. W., Bourcet, E. & Boyse, E. A. *J. exp. Med.* **157**, 1077–1088 (1983).
13. Harp, J. A. & Ewald, S. J. *Cell Immun.* **81**, 71–80 (1983).
14. Ralph, S. J. & Berridge, M. V. *J. Immun.* **132**, 2510–2514 (1984).
15. Sitkovsky, M., Pasternack, M. S., Lugo, J. P., Klein, J. R. & Eisen, H. N. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1519–1523 (1984).
16. Favero, J. J. *et al. Cell. Immun.* **68**, 38–52 (1982).
17. Nakayama, E., Shiku, H., Stoucent, E., Oettgen, H. F. & Old, L. J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1977–1981 (1979).

18. Harp, J. A., Davis, B. S. & Ewald, S. J. *J. Immun.* **133**, 10-15 (1984).
19. Minato, N., Reid, L., Cantor, H., Lengyel, P. & Bloom, B. R. *J. exp. Med.* **152**, 124-137 (1980).
20. Brooks, C. G., Kuribayashi, K., Sole, G. E. & Henney, C. S. *J. Immun.* **128**, 2326-2335 (1982).
21. Davignon, D., Martz, E., Reynolds, T., Kurzinger, K. & Springer, T. A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4535-4539 (1981).
22. Kimura, A. K. & Wigzell, H. *J. exp. Med.* **147**, 1418-1434 (1978).
23. Abo, T. & Balch, C. M. *J. Immun.* **127**, 1024-1029 (1981).
24. Kruse, J. *et al. Nature* **311**, 153-155 (1984).
25. Sarmiento, M., Loken, M. R., Trowbridge, I. S., Coffman, R. L. & Fitch, F. W. *J. Immun.* **128**, 1676-1684 (1982).
26. Hemler, M. E., Ware, C. F. & Strominger, J. L. *J. Immun.* **131**, 334-340 (1983).
27. Hemler, M. E. *et al. J. Immun.* **132**, 3011-3018 (1984).
28. Hubbard, A. L. & Cohn, Z. A. *J. Cell Biol.* **55**, 390-405 (1972).
29. Laemmli, U. K. *Natire* **227**, 680-685 (1970).

Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences

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The specificity of monoclonal antibodies provides a powerful diagnostic and therapeutic tool in investigating human neoplasia. Radiological scanning and immunotherapy with mouse tumour-specific monoclonal antibodies have been applied to patients with some success[1-3], but a major problem is the neutralization of the mouse antibody induced by repeated administration of heterologous antibodies. To avoid or reduce such immune reactions, chimaeric immunoglobulins consisting of mouse variable (V) and human constant (C) regions can be synthesized. We have constructed a recombinant retrovirus DNA carrying genomic heavy-chain (H) variable–diversity joining (V_H–D–J_H) and $C_\gamma 1$ genes from different species and show here that the chimaeric intervening sequences are spliced out precisely. This procedure provides a useful method to construct the chimaeric mouse–human immunoglobulin gene to be expressed in *Escherichia coli*, yeast and animal cells. Unexpectedly, a hidden splice donor site in the 5′-flanking region of a human V_H gene is used in place of the donor site of the leader sequence exon, resulting in the formation of the V region without the leader sequence.

We have chosen two pairs of immunoglobulin V_H and C_H genes from different species as models for the chimaeric recombinant immunoglobulin heavy-chain gene. One pair consists of the mouse $C_\gamma 1$ gene[4] and a human V_H gene encoding a V_H region against the surface antigen of hepatitis B virus (HBV). These two genes were inserted into a retrovirus vector plasmid, pTK-2ΔΔter (R)[5] and the recombinant virus plasmid abbreviated as pSNV$_1$·hV$_{HBV}$-mC$_\gamma$1 (Fig. 1*a*). The other pair, consisting of the human $C_\gamma 1$ gene[6] and the mouse V_H gene of TEPC15 myeloma[7], was inserted into pSNVΔTK (ref. 8) and the recombinant virus plasmid abbreviated as pSNV$_2$·mV$_{T15}$-hC$_\gamma$1 (Fig. 1*b*). These two vectors are derived from spleen necrosis virus (SNV). As the removal of the poly(A) addition signal AATAAA of an inserted foreign gene increases the recovery of the SNV vector[5], we removed the sequence 3′ to the *Hin*fI site located 39 base pairs (bp) downstream of the mouse $C_\gamma 1$ coding sequence and the sequence 3′ to the *Hha*I site located 88 bp downstream of the human $C_\gamma 1$ coding sequence.

To recover infectious viruses carrying the immunoglobulin gene, pSNV$_1$·hV$_{HBV}$-mC_γ1 and pSNV$_1$·mV$_{T15}$-hC_γ1 DNAs were used to transfect chicken embryo fibroblasts with or without 1/10th as much DNA of a provirus clone of a non-defective helper virus, reticuloendotheliosis virus strain A (REV-A)[9]. Before transfection, pSNV$_1$·hV$_{HBS}$-mC_γ1 DNA was digested with *Sal*I to remove the pBR322 sequence. Virus production was assayed by reverse transcriptase activity[10]. The enzyme activity of the culture supernatants of the cells transfected with recombinant and helper viral DNAs rose to ~10-fold higher than that of the background on day 5-7 after transfection. We next used viruses in culture supernatants recovered 7-10 days after transfection to infect fresh chicken embryo fibroblasts. Three days later, non-integrated linear viral DNA was isolated and examined for processing of the inserted DNA by Southern blot hybridization.

The parental pSNV$_1$·hV$_{HBV}$-mC_γ1 DNA contains the 3.6-kilobase (kb) *Bgl*II fragment that hybridizes to both human V_{HBV} and mouse $C_\gamma 1$ probes (probes A and B in Fig. 1*a*, respectively). On the other hand, *Bgl*II digestion of the processed pSNV$_1$·hV$_{HBV}$-mC_γ1 DNA should yield a 2.6-kb fragment hybridizing to both probes (Fig. 1). As shown in Fig. 2*a*, viral DNA from chicken embryonic fibroblasts transfected with pSNV$_1$·hV$_{HBV}$-mC_γ1 and helper virus DNA contained the 3.6- and 2.1-kb *Bgl*II fragments hybridizing to both human V_{HBV} and mouse $C_\gamma 1$ probes, suggesting that the viral DNA contains the parental and processed forms. No significant viral DNA was recovered from transfectants without the helper virus DNA (Fig. 2*a*, lanes 2, 3, 7, 8).

The parental pSNV$_2$·mV$_{T15}$-hC_γ1 DNA contains the 1.3-kb *Bam*HI fragment hybridizing to the mouse V_{T15} probe (probe C) and a 3.6-kb *Bam*HI fragment hybridizing to the human $C_\gamma 1$ probe (probe D). *Bam*HI digestion of the processed pSNV$_2$·mV$_{T15}$-hC_γ1 should yield a 1.7-kb fragment hybridizing both to probes C and D. Viral DNA isolated from transfectants by pSNV$_2$·mV$_{T15}$-hC_γ1 and helper viruses contained the 1.7- and 1.3-kb *Bam*HI fragments hybridizing to probe C (Fig. 2*b*, lanes 1-5) and the 1.7- and 3.6-kb *Bam*HI fragments hybridizing to probe D (Fig. 2*b*, lanes 6-10). There are three other potential donor splice sites (J_H2, J_H3 and J_H4) paired with the acceptor splice site of the C_H1 exon. If the donor sites of the J_H2, J_H3 and J_H4 segments were used, we should see the 2.2-, 1.5- and 2.0-kb *Bam*HI bands, respectively, hybridizing to the human $C_\gamma 1$ probe. There were no additional faint bands between 3.6- and 1.7-kb *Bam*HI bands, excluding these possibilities and suggesting that the chimaeric immunoglobulin genes are processed at the expected intron/exon splice sites.

To analyse the structure of the progeny viruses in more detail, the inserts of the progeny viruses were cloned into Charon 28H vector, a derivative of Charon 28 (see Fig. 3 legend). The progeny viral DNAs of pSNV$_1$·hV$_{HBV}$-mC_γ1 and pSNV$_2$·mV$_{T15}$-hC_γ1 were digested with *Bgl*II and *Bam*HI, respectively, and fractionated by agarose gel electrophoresis. DNA fractions containing the 2.1-kb *Bgl*II and 1.7-kb *Bam*HI fragments were ligated into the *Bam*HI site of phage Charon 28H DNA. Recombinant phages were screened by appropriate immunoglobulin gene probes (Fig. 3). Restriction enzyme cleavage analysis indicated that the eight hV$_{HBV}$-mC_γ1 clones and the two mV$_{T15}$-hC_γ1 clones obtained are identical with respect to restriction-site mapping.

The restriction map of the cloned hV$_{HBV}$-mC_γ1 DNA is consistent with that of the processed chimaeric gene of the human V_{HBV} and mouse $C_\gamma 1$ gene except that the left-most *Taq*I/*Xba*I is shortened by 500 bp (Fig. 1*a*). Disappearance of the *Bam*HI site in the 5′-flanking region of the processed hV$_{HBV}$-mC_γ1 agrees with the reduction of the *Taq*I/*Xba*I fragment size, suggesting that segmental deletion occurs in the 5′-flanking region of the V_{HBV} gene. The total length of the cloned hV$_{HBV}$-mC_γ1 insert is 2.1 kb, 500 bp shorter than predicted, in agreement with the size of the *Bgl*II fragment (2.1 kb) observed by Southern blot hybridization (Fig. 2*a*). The 500-bp deletion in *Taq*I/*Xba*I fragment, therefore, does not result from a cloning artefact but from genetic events during virus replication. Restriction-site mapping of the progeny mV$_{T15}$-hC_γ1 clones is consistent with the loss of all introns from the parental mV$_{T15}$-hC_γ1 insert.

Nucleotide sequences surrounding the junctions of the *V* and *C* gene sequences of the progeny clones are aligned with those around the splice sites of the parental *V* and *C* gene exons as shown in Fig. 3. It is clear that the *V*–*C* junctions of pSNV$_1$·hV$_{HBV}$-mC_γ1 and pSNV$_2$·mV$_{T15}$-hC_γ1 are processed precisely according to the GT-AG rule[11], indicating that interspecies pairs of exons easily allow precise removal of introns.

lucleotide sequences of other exon junctions also demonstrate iat all the introns are spliced out exactly as expected (data not iown).

We also read the sequence of the 5′-flanking region of the enomic V_{HBV} gene and find that there is a potential donor splice site, CCGGTGAGA, at 268–276 bp downstream from the *Taq*I site. Comparison of the nucleotide sequences of the parental and progeny V_{HBV} genes and their upstream regions reveals that the region extending from this potential donor site to the acceptor site of the V_{HBV} exon is removed (Fig. 3). Therefore,

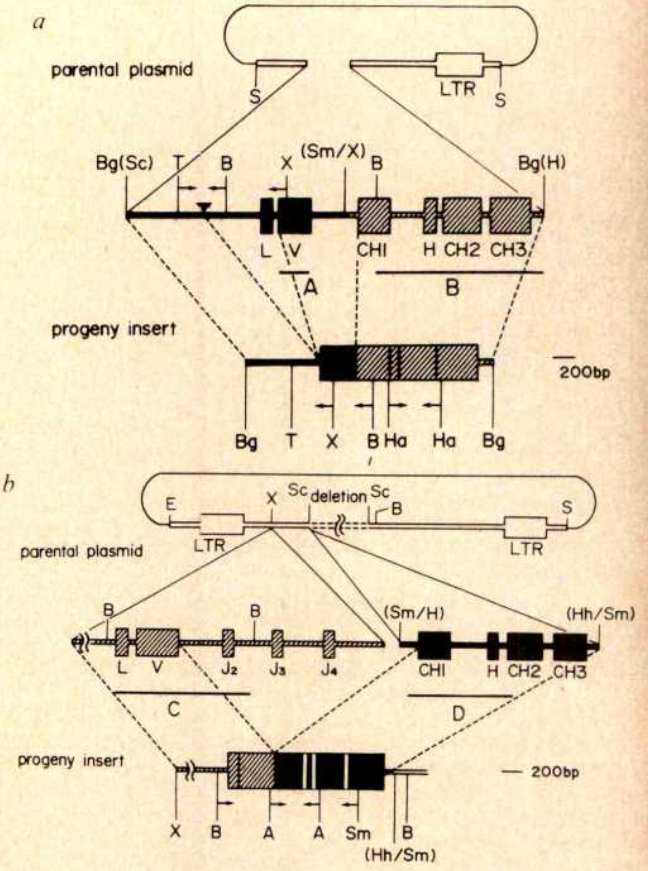


Fig. 1 Structure of parental and progeny recombinant immunoglobulin genes; *a*, $pSNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$; *b*, $pSNV_2 \cdot mV_{T15}\text{-}hC_\gamma 1$. Parental recombinant plasmids containing retroviral and immunoglobulin sequences are shown at the top and immunoglobulin gene sequences in progeny retroviruses at the bottom. Retrovirus, mouse and human immunoglobulin sequences are indicated by open, oblique and solid rectangles, respectively. The long terminal repeat (LTR) and exons are shown by wider rectangles and the pBR322 sequence by a solid line. Corresponding DNA segments in parental and progeny viruses are aligned by dotted lines. Solid bars indicate probes used. Directions and ranges of sequences read are shown by horizontal arrows. L, leader exon; V, *V–D–J* exon; H, hinge exon; C_H1, C_H2 and C_H3, exons encoding each domain. Restriction sites are indicated as follows: A, *Ava*II; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*fI; Ha, *Hae*III; Hh, *Hha*I; S, *Sal*I; Sc, *Sac*II; Sm, *Sma*I; T, *Taq*I; X, *Xba*I. In *a*, fragments A and B indicate the *Sau*3A/*Hha*I fragment of the human V_{HBV} gene and the *Bam*HI/*Bgl*II fragment of the mouse $C_\gamma 1$ gene, respectively. The closed triangle indicates the hidden donor splice site in the *Taq*I/*Bam*HI fragment. In *b*, fragments C and D indicate the *Bam*HI fragment of the mouse V_{T15} gene and the *Sac*II fragment of the human $C_\gamma 1$ gene, respectively.

Methods. Construction of $pSNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$. We cloned the V_H gene encoding the V region against the surface antigen of HBV from the Epstein-Barr-virus-transformed cell and determined the nucleotide sequence of the coding region (T. Noma and T. Honjo, unpublished). The 2.2-kb (*Sac*II/*Sma*I) fragment of the human V_{HBV} gene was prepared. The *Sac*I fragment (2.5 kb) including the mouse $C_\gamma 1$ gene sequence was isolated electrophoretically from λgtWES·IgH-2 (ref. 4) and digested separately with *Xba*I and *Sma*I, and with *Sma*I and *Hin*fI. *Hin*fI digests the $C_\gamma 1$ gene 30 bp upstream from the poly(A) addition signal AATAAA. We inserted the 2.2-kb *Sac*II/*Sma*I fragment of the human V_{HBV} gene and the 0.88-kb *Xba*I/*Sma*I and 0.64-kb *Sma*I/*Hin*fI fragments of the mouse $C_\gamma 1$ gene into the *Bgl*II site of pTK-2ΔΔTer (R) (ref. 5). The *Xba*I site of the mouse $C_\gamma 1$ gene was treated with the Klenow fragment of DNA polymerase I to make the blunt end and ligated with the *Sma*I site of the human V_{HBV} gene. The *Sac*II site of human V_{HBV} and the *Hin*fI site of mouse $C_\gamma 1$ gene were filled with Klenow fragment and ligated with *Bgl*II linker (Takara Co. Ltd). The intron between the *V* and *C* exons consists of 460 bp of 3′-noncoding sequence of the V_{HBV}–D–J_H gene and 50 bp of the 5-noncoding sequence of the mouse $C_\gamma 1$ gene. Construction of $pSNV_2 \cdot mV_{T15}\text{-}hC_\gamma 1$. pSNV-TK (ref. 8) was digested with *Sac*II to remove the 1.6-kb *Sac*II fragment in the *tk* gene and then recircularized with T4 ligase to give plasmid pSNVΔTK. The *Hin*dIII fragment of $Ig_\gamma 1$-10-containing human $C_\gamma 1$ gene[6] was first subcloned into pBR322 and the plasmid then digested separately with *Hin*dIII and *Pst*I, and with *Pst*I and *Hha*I. (*Hha*I cleaves the $C_\gamma 1$ gene 15 bp upstream from AATAAA sequence.) Equimolar amounts of the *Hin*dIII/*Pst*I fragment containing the C_H1 exon and the *Pst*I/*Hha*I fragment containing the C_H2, hinge and C_H3 exons were ligated together. The 1.9-kb fragment corresponding to the length of the intact *Hin*dIII/*Hha*I fragment of the human $C_\gamma 1$ gene was purified and the ends of the fragment were filled by the Klenow fragment of DNA polymerase I. The blunt-ended DNA fragment was inserted into the *Sma*I site of pSNVΔTK and the *Xba*I fragment containing the V_{T15} sequence[7] then inserted into the *Xba*I site of $pSNV\Delta TK \cdot C_\gamma 1$. The intron between the V_{T15} and $C\gamma 1$ exons consists of the 3′-noncoding region of the V_{T15} (2 kb) containing the J_H2, J_H3 and J_H4 exons, the 5′-noncoding region of the human $C_\gamma 1$ gene(200 bp) and the *Xba*I/*Sma*I fragment of pSNV-TK (200 bp).

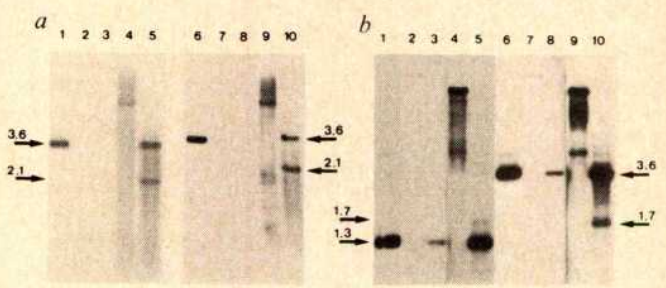


Fig. 2 Structural analyses of nonintegrated progeny viral DNA from chicken fibroblasts infected with recombinant viruses. Non-integrated viral DNA was analysed by Southern blot hybridization using the nick-translated probes indicated. *a*, Progeny virus of $pSNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$. DNA is as follows: lanes 1, 6, parental $pSNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$; lanes 2, 3, 7, 8, progeny of $pSNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$ recovered from chicken fibroblast cells transfected without helper virus DNA; lanes 4, 5, 9, 10, progeny of $pSNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$ recovered from fibroblasts transfected with helper virus DNA. DNAs in lanes 1, 3, 5, 6, 8 and 10 were digested with *Bgl*II. Hybridization probes used: lanes 1–5, probe A; lanes 6–10, probe B described in Fig. 1. *b*, Progeny virus of $pSNV_2 \cdot mV_{T15}\text{-}hC_\gamma 1$. DNA lanes 1, 6, parental $pSNV_2 \cdot mV_{T15}\text{-}hC_\gamma 1$; lanes 2, 3, 7, 8, progeny of $pSNV_2 \cdot mV_{T15}\text{-}hC_\gamma 1$ recovered from fibroblasts transfected without helper virus DNA; lanes 4, 5, 9, 10, progeny of $pSNV_2 \cdot mV_{T15}\text{-}C_\gamma 1$ recovered from fibroblasts transfected with helper virus DNA. DNAs in lanes 1, 3, 5, 6, 8 and 10 were digested with *Bam*HI. Hybridization probes used: lanes 1–5, probe C; lanes 6–10, probe D described in Fig. 1. Lengths of hybridized bands are given in kb; arrows indicate the bands derived from the parental or processed hybrid immunoglobulin gene.

Methods. The 8.2-kb *Sal*I fragment of $pSNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$ was isolated by agarose gel electrophoresis and ligated with T_4 DNA ligase at 15 °C for 10 h. Homopolymeric $SNV_1 \cdot hV_{HBV}\text{-}mC_\gamma 1$ and circular $pSNV_2 \cdot mV_{T15}\text{-}hC_\gamma 1$ DNA (10 μg each) were introduced separately into chicken fibrobalsts in a tissue-culture flask (25 cm^2) in the presence or the absence of REV-A DNA (1 μg) by the calcium phosphate co-precipitation technique[17]. Virus production was assayed by the reverse transcriptase activity[10]. After 7–10 days, the tissue culture medium containing the recombinant virus was collected and fresh chicken cells in a 100-mm Petri dish were infected with undiluted virus. Three days after infection, non-integrated viral DNA was isolated by the Hirt procedure from the infected cells[18,19]. The non-integrated viral DNAs were digested with *Bgl*II or *Bam*HI and analysed by electrophoresis in a 0.8% agarose gel. Each lane contained DNA from one 100-mm Petri dish. The gel was blotted onto nitrocellulose filter paper and the filter hybridized with the nick-translated ^{32}P-labelled probe indicated.

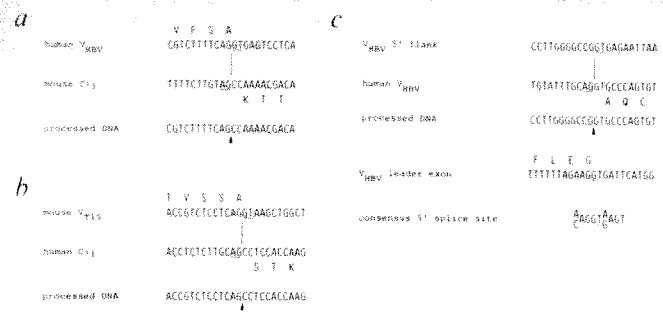


Fig. 3 Nucleotide sequences surrounding processed exon junctions in progeny viruses. *a*, Sequence surrounding the V_{HBV} and mouse C_H1 exon junction in progeny virus of $pSNV_1 \cdot V_{HBV}$-$mC_\gamma1$. *b*, Sequence surrounding the V_{T15} and human C_H1 exon junction in progeny virus of $pSNV_2 \cdot mV_{T15}$-$hC_\gamma1$. *c*, Sequence surrounding the recombination site between the potential donor splice site in the 5′-flanking region of the V_{HBV} gene and the V_{HBV} exon. Nucleotide sequences around the exon/intron junctions in parental virus are shown above the sequence of processed gene. The consensus sequence of the donor splice site[12] and the 5′ splice site of leader sequence of V_{HBV} are also shown. GT and AG are underlined. Splice sites are designated by arrowheads. Nucleotide sequences determined (~200 bp at each junction) are identical to those predicted from parental gene sequences taken from the literature[4,20,21]. **Method.** Virus-infected chicken cells in 12 100-mm Petri dishes were lysed with SDS 3 days after infection. Non-integrated viral DNA was isolated by Hirt's procedure[18,19]. The 1.7-kb *Bam*HI fragment containing the processed mV_{T15}-$hC_\gamma1$ gene and the 2.1-kb *Bgl*II fragment containing the processed hV_{HBV}-$mC_\gamma1$ gene were isolated by agarose gel electrophoresis. These fragments were inserted separately into Charon 28H, a modified Charon 28 vector (described below), with T_4 ligase and packaged *in vitro*. Recombinant phages containing hV_{HBV}-$mC_\gamma1$ and mV_{T15}-$hC_\gamma1$ were screened using probes A and C, respectively. We identified eight phage clones that contained the hybrid hV_{HBV}-$mC_\gamma1$ gene fragment and two phage clones that contained the hybrid mV_{T15}-$hC_\gamma1$ gene fragment. There is an *Eco*RI site in each arm of Charon 28H. We purified the *Eco*RI fragment containing processed hV_{HBV}-$mC_\gamma1$ gene and the *Bam*HI fragment containing processed mV_{T15}-$hC_\gamma1$ gene for restriction enzyme analysis and sequencing. The *Eco*RI fragment containing hV_{HBV}-$mC_\gamma1$ and the *Bam*HI fragment containing mV_{T15}-$hC_\gamma1$ were cleaved with restriction enzymes shown in Fig. 1 and their ends were labelled by polynucleotide kinase. Labelled fragments were isolated and sequenced according to Maxam and Gilbert[22]. We also sequenced the *Taq*I/*Xba*I fragment of the 5′-flanking region of the V_{HBV} gene. Construction of Charon 28H vector. The 6.8-kb *Bam*HI fragment of mouse immunoglobulin $S_\gamma2a$ region gene[23] was cloned in pBR322. The recombinant plasmid was digested with *Bgl*II and inserted into *Bam*HI site of Charon 28 (ref. 24) to construct Charon 28H vector. Charon 28H DNA was digested with *Bam*HI and *Sal*I and the internal fragment derived from pBR322 was removed to prepare arms. Longer and shorter arms of the modified Charon 28H are 4.1 and 2.7 kb, respectively, longer than the original Charon 28.

there is preference of the 5′ potential splice site over the normal donor site of the signal peptide exon. The nucleotide sequences of both the 5′ potential and normal donor sites mismatch at two bases with the consensus 9-base sequence surrounding the donor splice site[12]. Except for GGTGA sequence, there is little sequence homology between the nucleotide sequences surrounding the potential and normal donor sites. There are several examples where mutations in normal splice donor sites have revealed potential donor splice sites in exons[13–16]. The presence of a donor splice site more potent than the normal donor site in the flanking region has not been reported.

We have shown here that the strategy using the retrovirus vector is useful for construction of hybrid immunoglobin genes and will eventually enable expression of the hybrid protein in yeast, *E. coli* and animal cells. Our results demonstrate that the genomic *V* and *C* exons derived from different species are properly fused. We have used the same strategy to construct the hybrid light-chain genes composed of mouse V_K and human C_K genes. Most clinically useful antibodies are made more easily in mouse and are difficult to administer in humans. If the C region of the mouse monoclonal antibody against human cancer cells could be replaced by a human immunoglobulin C region, the clinical advantages would include the facts that the hybric immunoglobulin is less antigenic and the most useful C_H regior could be chosen for treatment.

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1. Ritz, J. & Shlossman, S. F. *Blood* **59**, 1–11 (1982).
2. Ritz, J. *et al. Blood* **58**, 141–152 (1981).
3. Miller, R. A., Maloney, D. G., Warnke, R. & Levy, R. *New Engl. J. Med.* **306**, 517–522 (1982).
4. Honjo, T. *et al. Cell* **18**, 559–568 (1979).
5. Shimotohno, K. & Temin, H. M. *Cell* **26**, 67–77 (1981).
6. Takahashi, N. *et al. Cell* **29**, 671–679 (1982).
7. Nakanishi, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6984–6988 (1982).
8. Shimotohno, K. & Temin, H. M. *Nature* **299**, 265–268 (1982).
9. O'Rear, J. J. & Temin, H. M. *J. Virol.* **39**, 138–149 (1981).
10. Hagino, K., Kano, K., Ikegami, S., Nagano, H. & Mano, Y. *Virology* **107**, 174–179 (1980).
11. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4853–4857 (1978).
12. Cech, T. R. *Cell* **34**, 713–716 (1983).
13. Seidman, J. G. & Leder, P. *Nature* **286**, 779–783 (1980).
14. Choi, E., Kuehl, M. & Wall, R. *Nature* **286**, 776–779 (1980).
15. Wieringa, B., Meyer, F., Reiser, J. & Weissmann, C. *Nature* **301**, 38–43 (1983).
16. Treisman, R., Orkin, S. & Maniatis, T. *Nature* **302**, 591–596 (1983).
17. Graham, F. L. & Van der Eb, A. J. *Virology* **52**, 456–467 (1973).
18. Hirt, B. *J. molec. Biol.* **26**, 365–369 (1967).
19. Chen, I. S. Y. & Temin, H. M. *J. Virol.* **41**, 183–191 (1982).
20. Ellison, J. W., Berson, B. J. & Hood, L. E. *Nucleic Acids Res.* **10**, 4071–4079 (1982).
21. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. *Cell* **19**, 981–992 (1980).
22. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
23. Nikaido, T., Yamawaki-Kataoka, Y. & Honjo, T. *J. biol. Chem.* **257**, 7322–7329 (1982).
24. Liu, C.-P., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. *Science* **209**, 1348–1353 (1980).

Thymic epithelium and the induction of transplantation tolerance in nude mice

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Immunological tolerance of self has been studied experimentally by the induction of unresponsiveness to antigens of the major histocompatibility complex (MHC) in neonatal mice[1]. The specific unresponsiveness resulting from such neonatal tolerance induction is first demonstrable in the thymus[2,3], suggesting that neonatal tolerance is induced by some cellular component of the thymus, or at some prethymic stage. Recent transplantation studies suggest that thymic epithelium, derived by organ culturing fetal mouse thymus in the presence of deoxyguanosine, survives in an allogeneic host environment despite the continued expression of MHC donor antigens, but fails to induce allotolerance[4]. We demonstrate here that embryonic thymus lobes organ cultured at 24°C, a treatment that deletes the lymphohaematopoietic component of thymus leaving the epithelial matrix intact[5], when transplanted to intact histoincompatible recipients, similarly survive for a prolonged period and do not induce tolerance to donor MHC antigens. However, when such culture-derived thymic epithelium is allografted to athymic nude mice, host-derived lymphocytes from both the epithelial graft and recipient spleen are unresponsive to the MHC antigens of the epithelial donor. The results suggest that, when investigated in a system which precludes the possible involvement of extrinsic mature T cells, processed by the syngeneic host thymus, thymic epithelium may induce transplantation tolerance.

Low-temperature (24 °C) organ culture was first used to deplete passenger leukocytes in studies of pancreatic islet

Table 1 Effect of low-temperature organ culture on the survival of fetal thymus grafts in allogeneic recipients

Duration of grafts (days)	No. of animals with surviving grafts: Low-temperature culture (24 °C)	Conventional culture (37 °C)	Uncultured 14-day thymus
5	5/5	5/5	5/5
10	5/5	0/5	3/5
15	5/5	0/5	1/5
25	5/5	0/5	0/5
40	3/5	0/5	0/5
50	1/5	0/5	0/5

Fetal thymus lobes were isolated from C57BL/6 (H-2^b) embryos on day 14 of gestation and organ cultured for 8 days at either 24 or 37 °C as described previously[6]. Two lobes were transplanted beneath the renal capsule of C_3H (H-2^k) recipients. Uncultured lobes were transplanted to allogeneic recipients immediately following isolation from 14-day donor embryos. Host mice were killed at varying times post-grafting (5–50 days) and the presence or absence of grafts was scored using a dissecting microscope. Successful grafts were readily identified as fleshy, white subcapsular swellings. The absence of grafts was always confirmed histologically. Grafts of fetal thymus, whether low-temperature organ cultured or not, survived indefinitely in syngenic recipients of allogeneic (H-2^d) nude mice (data not shown). The results show that, whilst untreated 14-day thymic lobes and those organ cultured at 37 °C are acutely rejected by allogeneic hosts, low-temperature (24 °C) cultured grafts survive for prolonged periods.

allograft survival[6] and has been shown subsequently to delete selectively the lymphoid component of fetal mouse thymus, leaving the epithelial matrix intact[5,7]. When 14-day fetal thymus lobes, cultured for 8 days at 24 °C, are grafted beneath the renal capsule of intact syngeneic mice, the surviving thymic epithelial matrix is colonized by host-derived lymphoid precursors which differentiate into thymocytes, thus forming an heterotopic lymphoid thymus[5,8]. The regenerating intragraft lymphocyte population is 95% Thy-1^+, 80% TL^+, 95% Lyt 1^+, 90% Lyt 2^+ and includes a cortisone-resistant functional subpopulation responsive to alloantigens in mixed lymphocyte culture (MLC) as well as to T-cell mitogens (refs 8, 9 and R.K.J. and N.A.H., unpublished data).

It has been reported previously that embryonic thymus lobes depleted of their endogenous lymphophaematopoietic cells by deoxyguanosine treatment *in vitro* can be successfully transplanted across major histocompatibility barriers, even though the surviving thymic epithelium continues to express class II MHC antigens[4]. To determine whether low-temperature organ culture would allow the similar survival of fetal thymic allografts, C3H (H-2^k) mice were grafted with thymus lobes from C57BL/6 (H-2^b) embryos of 14 days' gestation, organ cultured for 8 days at 24°C. Survival of these experimental grafts was compared with that of 14-day fetal thymic lobes with and without conventional organ culture at 37 °C. Table 1 shows that, whereas uncultured and conventionally cultured grafts are rejected normally by 15 days post-grafting, fetal thymus lobes cultured at 24 °C escape this acute rejection. Survival of these thymic epithelial allografts, at least in this strain combination (C57BL/6 into C3H), is not indefinite. Although 100% of low-temperature treated grafts were recovered at 25 days, this proportion fell to 60% by 40 days and to 20% by 50 days. Surviving allogeneic epithelial grafts seem to grow in the same way as they do in syngeneic recipients, demonstrating organotypic thymic development and giving a maximal lymphoid cell yield of about 5×10^6 per lobe.

The probable explanation for the observed prolongation of allograft survival is that the low-temperature treatment of fetal thymus not only eliminates the endogenous thymocytes and their progenitors, but also substantially reduces or even deletes the donor class II MHC-antigen-expressing dendritic cells of the thymus, thereby avoiding route 1 rejection as described by Lechler and Batchelor[10]. Thus, when allografted, the thymic epithelium derived by low-temperature organ culture seems to behave in a similar fashion to that obtained by deoxyguanosine treatment[4], except that, over the longer timescale reported here, low-temperature-treated grafts seem eventually rejected. This late rejection may reflect the survival of small numbers of donor-derived dendritic cells[11,12] of haematogenous origin within the graft or it may be a manifestation of the alternative rejection route proposed by Lechler and Batchelor[10] by which graft antigens are processed and presented by the recipients' own accessory cells.

We confirm the findings of Ready *et al.*[4] that lymphocytes recovered from both the epithelial grafts and the spleens of intact allogeneic recipients respond to the MHC antigens of the thymic donor type in MLC (Table 2), arguing against a role for thymic epithelium in the induction of tolerance. However, as the recipients of the thymic epithelial grafts were normal intact histoincompatible mice, it cannot be concluded necessarily that the reactive T cells present in the grafts had, in fact, matured there. Indeed, it is possible that the reactive T cells had matured in the syngeneic recipient thymus and subsequently migrated into the allogeneic thymic epithelial graft. To eliminate this possibility, we also transplanted low-temperature organ-culture-derived thymic epithelium to athymic nude mice, where there was no possibility of host thymic processing.

Athymic BALB/c *nu/nu* (H-2^d) mice were grafted with low-temperature-treated C57BL/6 (H-2^b) fetal thymic lobes. Such

Table 2 MLC responses of spleen and thymus graft cells from C_3H(H-2^k) mice bearing low-temperature organ-cultured C57BL/6 (H-2^b) thymus grafts beneath the renal capsule

Animal no.	Responders	Stimulators: Host type (H-2^k)	Graft type (H-2^b)	Third party (H-2^d)	*P* values
1	Intragraft lymphocytes	564 ± 91	4,815 ± 736	5,392 ± 124	<0.01; <0.001
	Host spleen	16,006 ± 1,079	38,048 ± 312	49,546 ± 1,318	<0.001; <0.001
2	Intragraft lymphocytes	475 ± 40	2,368 ± 178	1,517 ± 231	<0.001; <0.05
	Host spleen	7,807 ± 673	18,780 ± 52	24,250 ± 295	<0.001; <0.001
3	Intragraft lymphocytes	362 ± 40	3,364 ± 143	2,454 ± 155	<0.001; <0.001
	Host spleen	NT	NT	NT	

MLC assays were performed in U-well microtitre plates. Responder cells were prepared by teasing host spleen and thymus grafts and were used at a final concentration of 2×10^5 per well. Stimulators were mitomycin-C-treated (30 μg ml^{-1} for 40 min at 37 °C) spleen cells titrated over the range 4×10^5–2.5×10^4 per well. All cultures were performed in 200 μl of supplemented RPMI 1640 medium with 10% fetal calf serum. Cultures were pulsed with 1 μCi ml^{-1} of ^{3}H-thymidine for 18 h on day 4 of culture and collected with an automatic sample collector. Results are expressed as the mean d.p.m. ± s.e.m. for three replicate wells at the optimal stimulator: responder ratio. *P* values are given for the response to graft-type and third-party stimulators as compared with the response to host-type stimulators. The results show that there are significant responses to both graft-type and third-party stimulators by cells from both host spleen and thymus grafts. NT, not tested.

Table 3 MLC responses of spleen and thymus graft cells from athymic BALB/c (H-2^d) nude mice bearing low-temperature organ culture C57BL/6 (H-2^b) thymus grafts beneath the renal capsule

Animal no.	Responders	Stimulators: Host type (H-2^d)	Graft type (H-2^b)	Third party (H-2^k)	*P* values
1	Intragraft lymphocytes	412 ± 95	326 ± 102	4,338 ± 59	NS; <0.001
	Host spleen	12,261 ± 1,471	16,672 ± 896	25,614 ± 2,348	NS; <0.01
2	Intragraft lymphocytes	258 ± 60	243 ± 53	2,779 ± 159	NS; <0.001
	Host spleen	13,212 ± 1,294	14,417 ± 796	24,212 ± 1,261	NS; <0.01
3	Intragraft lymphocytes	468 ± 114	859 ± 246	2,050 ± 244	NS; <0.01
	Host spleen	16,438 ± 2,615	17,046 ± 1,050	28,793 ± 2,150	NS; <0.01

MLC cultures were carried out as described in Table 2 legend. The results show that while there are significant responses to third-party stimulators by cells from both nude host spleen and thymus grafts, both populations failed to respond to graft-type stimulators. NS, not significant.

thymic epithelial grafts, placed beneath the renal capsule, became recolonized by T-cell precursors to form a fully lymphoid heterotopic thymus. The host derivation of the repopulating lymphoid component was confirmed using monoclonal anti-D^d (supernatant from clone 34-4-21s) and anti-K^b (supernatant from clone 28-13-3s) antibodies (data not shown). By 56 days post-grafting, the peripheral T-cell compartment of the nude recipients was significantly reconstituted as judged by the number of Thy-1^+ cells in the host spleen and the restoration of responsiveness to the T-cell mitogen concanavalin A (Con A) (Table 4). In these nude recipients, we found that host-derived lymphocytes (H-2^d) within the epithelial graft respond in MLC (Table 3) to the MHC antigens of a third party (H-2^k), but not to the MHC antigens of the thymic epithelial donor (H-2^b). Table 3 also shows that spleen cells from the recipient nudes respond to third-party stimulators (H-2^k) but not to graft type (H-2^b); however, the high level of proliferation to host type (H-2^d) obscures a clear demonstration of either reconstitution of the MLC response or tolerance in the spleen population.

Thus, in the nude thymic chimaera, the low-temperature-cultured thymic allografts can be shown to induce tolerance while failing to do so in normal thymus-intact recipients. The simplest explanation for this paradox is that the T cells reactive to donor graft type in the intact chimaera had matured in the syngeneic host thymus and migrated to the allogeneic thymic graft. That mature T cells can indeed recirculate to the normal thymus has been suggested previously by migration studies with labelled effector T-lymphocyte lines[13]. The results suggest that the epithelium remaining in low-temperature cultured grafts can induce tolerance, but that in allogeneic normal recipients this tolerance is masked.

That it is the grafted thymic epithelium which leads to tolerance induction is at this time only a probability, for the involvement of thymic dendritic cells cannot be entirely ruled out. We have been unable to isolate dendritic cells from low-temperature thymic organ cultures before grafting[8], but it cannot be precluded that the low-temperature treatment only reduces the number of dendritic cells rather than eliminating them altogether. However, as low-temperature-treated thymic allografts survive in thymus-intact recipients long after the stage of acute rejection, the presence of functionally significant numbers of dendritic cells is doubtful.

Although there is little question that the tolerance to donor MHC antigens observed in both the grafts and spleens of the nude recipients was induced by the presence of the low-temperature organ-cultured thymic lobes, the present study sheds little light on the nature of the tolerogenic mechanism. While these results are in keeping with the hypothesis that tolerance results from the deletion of clones reactive to graft type[2], the unresponsiveness to donor MHC antigens could be mediated by specific suppressor T cells, as demonstrated for classical neonatal tolerance[14,15].

In conclusion, we suggest that it is premature to exclude thymic epithelium from having a determining effect in tolerance induction, as our results can be interpreted as suggesting that thymic epithelial cells, which are known to express class II MHC antigens[16,17], may have a role in the induction of tolerance in helper T lymphocytes. Whether cultured thymic epithelium tolerizes cytotoxic T lymphocytes remains to be determined, but in this context it is interesting that the thymus appears to impose MHC restriction only for class II- and not for class I-restricted T-cell recognition[18,19].

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Note added in proof: It has recently been shown that thymocytes from deoxyguanosine-treated fetal thymi grafted to allogeneic nude recipients contain cytolytic T-lymphocyte precursors reactive to class I MHC antigens of thymic donor type[20].

Table 4 Thy-1 expression and Con A responsiveness of spleen lymphocytes recovered from BALB/c nude mice with and without C57BL/6 thymic epithelial grafts

Recipient	Graft	No. Thy 1.2 (CI ± s.e.m.)	Con A ($\bar{x}\Delta$ d.p.m. ± s.e.m.)
nu/+ (27)	—	28.2 ± 4.2	61,067 ± 6,427
nu/*nu* (12)	—	0.1 ± 0.6	370 ± 1,354
nu/*nu* (10)	+	9.6 ± 1.5	33,118 ± 6,922

CI, cytotoxic index, using anti-mouse Thy 1.2 (clone 30-H12, Becton-Dickinson) at 1 in 800 and rabbit complement (Low Tox M, Cedarlane) at 1 in 15. Viability was assessed by trypan blue dye exclusion and a total of 400 cells counted. For Con A experiments, 200-μl microcultures in triplicate using 2×10^5 spleen cells and 2 μg ml^{-1} Con A (Miles-Yeda) were incubated for 72 h with a pulse of 1 μCi ml^{-1} for the final 18 h. Numbers in parentheses are the number of mice tested.

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1. Billingham, R. E., Brent, L. & Medawar, P. B. *Nature* **172**, 603-606 (1953).
2. Nossal, G. J. V. & Pike, B. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3844-3847 (1981).
3. Wood, P. J. & Streilein, J. W. *Eur. J. Immun.* **12**, 188-194 (1982).
4. Ready, A. J., Jenkinson, E. J., Kingston, R. & Owen, J. J. T. *Nature* **310**, 231-233 (1984).
5. Jordan, R. K. & Crouse, D. A. in *Development and Differentiation of Vertebrate Lymphocytes* (ed. Horton, J. D.) 47-61 (Elsevier, Amsterdam, 1980).
6. Lacy, P. E., Davie, J. M. & Finke, E. H. *Science* **204**, 312-313 (1979).
7. Robinson, J. H. & Jordan, R. K. *Immun. Today* **4**, 41-45 (1983).
8. Jordan, R. K., Bentley, A. L., Perry, G. A. & Crouse, D. A. *J. Immun.* (in the press).
9. Jordan, R. K., Robinson, J. H., Bentley, A. L., House, K. C. & Hopkinson, N.A. in *Proc. 8th int. Conf. Lymphatic Tissues and Germinal Centers in Immune Reactions* (ed. Klauss, G. G. B.) (Plenum, New York, in the press).
10. Lechler, R. I. & Batchelor, J. R. *J. exp. Med.* **155**, 31-41 (1982).
11. Robinson, J. H. *J. Immun.* **130**, 1592-1595 (1983).
12. Barclay, A. N. & Mayrhofer, G. J. *J. exp. Med.* **153**, 1660-1671 (1981).
13. Fink, P. J., Bevan, M. J. & Weissman, I. L. *J. exp. Med.* **159**, 436-451 (1984).
14. Dorsch, S. & Roser, B. *Transplantation* **33**, 518-524 (1982).
15. Stockinger, B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 220-223 (1984).
16. Natali, P. G., de Martino, C., Pellegrino, M. A. & Ferrone, S. *Scand. J. Immun.* **13**, 541-546 (1981).
17. Jenkinson, E. J., van Ewijk, W. & Owen, J. J. T. *J. exp. Med.* **153**, 280-292 (1981).
18. Bradley, S. M., Kruisbeek, A. M. & Singer, A. *J. exp. Med.* **156**, 1650-1664 (1982).
19. Kast, W. M., de Waal, L. P. & Melief, J. M. *J. exp. Med.* **160**, 1752-1766 (1984).
20. von Boehmer, H. & Schubiger, K. *Eur. J. Immun.* **14**, 1048-1052 (1984).

Activation of major histocompatibility complex class I mRNA containing an *Alu*-like repeat in polyoma virus-transformed rat cells

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Class I genes of the major histocompatibility complex (MHC) appear to be activated in mouse cells transformed by the DNA tumour virus simian virus 40 (SV40)[1]. Conversely, suppression of MHC class I genes has been reported in adenovirus-12-transformed baby kidney rat cells[2]. We have now investigated the expression of genes encoded by the rat MHC locus in rat fibroblast cells transformed by polyoma virus (Py). Using a mouse genomic *H-2* clone as a probe in Northern transfer hybridization analysis, we have observed a high level of expression of rat MHC class I messenger RNA in all the transformed rat cell lines analysed. The class I 1.6-kilobase (kb) mRNA activated in Py-transformed rat cells appears to contain an *Alu*-like type II repeat element, as the same 1.6-kb mRNA is detected using either the *H-2* class I sequence or a repetitive *Alu*-like type II element as a probe. High levels of heterogeneous poly(A)$^+$ transcripts of 0.5–0.8 kb are also observed in Py-transformed rat cells using probes containing an *Alu*-like type II repetitive element.

The rat major histocompatibility complex, the *RT.1* complex, comprises the loci encoding the histocompatibility antigens[3]. Three loci, *RT.1 A* (analogues of mouse *H-2K* and *H-2D*)[4], *RT.1 E* (ref. 5) and *RT.1 C* (*Qa* analogue)[6], control the expression of the classical serologically defined class I antigens. Taking advantage of the relatively conserved nature of portions of mammalian MHC genes, we have used a mouse genomic *H-2* DNA probe to investigate the level of *RT.1* class I mRNA expression in Py-transformed rat cells and the parental untransformed cells. Figure 1 describes the structure of the mouse *H-2* clone pL4-7. The 2.7-kb *Bam*HI fragment isolated from a genomic library constructed from mouse DBA/2 seems to be similar, if not identical, to the 3'-end portion of the *27.1* gene isolated by Steinmetz *et al.*[7] from a BALB/c DNA library. As the probe contains the constant (third) domain, it should react with any MHC class I heavy-chain mRNA present in rat cells.

A series of Py-transformed rat cell lines was used to demonstrate the general applicability of the observed phenomena (Table 1). The Rat-1 (ref. 8) parental cell line and the Ag4-rat revertant cell line R3-rat, which has lost the integrated Py early DNA coding sequence (G.L.M. and L.L., unpublished results), were used as untransformed control cells.

Polyadenylated cytoplasmic RNAs from parental, transformed and revertant normal cells were electrophoresed in formaldehyde/agarose gel, transferred to nitrocellulose filter and hybridized with ^{32}P-labelled pL4-7 plasmid DNA (Fig. 2). All the Py-transformed rat cells analysed (Table 1), regardless of their origin, expressed a high level of mRNA of 1.6 kb and a heterogeneous class of transcripts of 0.5–0.8 kb (Fig. 2 and data not shown). The various Py-rat cell lines analysed seemed to express slightly different patterns of the heterogeneous small RNAs. Conversely, the untransformed parental Rat-1 and the normal revertant cells (Fig. 2, lanes 1, 2) expressed a very low level of 1.6-kb mRNA, which was almost undetectable after long exposure of these autoradiograms (2 weeks at −80 °C). The heterogeneous class of RNA transcripts of 0.5–0.8 kb was observed in the normal cells, although at a lower level in Rat-1 cells. Attempts to resolve the hybridization signal observed at 0.5–0.8 kb into discrete bands using different gel concentrations and electrophoresis conditions were unsuccessful.

These results indicate that a high level of expression of MHC class I mRNA is a common characteristic of Py-transformed rat cells, extending to the Py-rat system the notion originally proposed[1] that activation of class I major histocompatibility antigen genes is a general feature of oncogenesis in the mouse. However, the activation of MHC class I gene expression in Py-rat does not appear to be a general phenomenon of transformed rat cells, as it has been reported[2,9] that a MHC gene, *RT.1 A*^{u}, is switched off in adenovirus-12-transformed primary baby rat kidney cells.

To rule out the possibility that the high level of expression of *RT.1* class I mRNA in Py-rat cells is a result of extensive amplification of the corresponding *RT.1* genomic sequences, we have used the Southern[10] hybridization technique to analyse the structure of the *RT.1* locus in Py-rat and in the normal

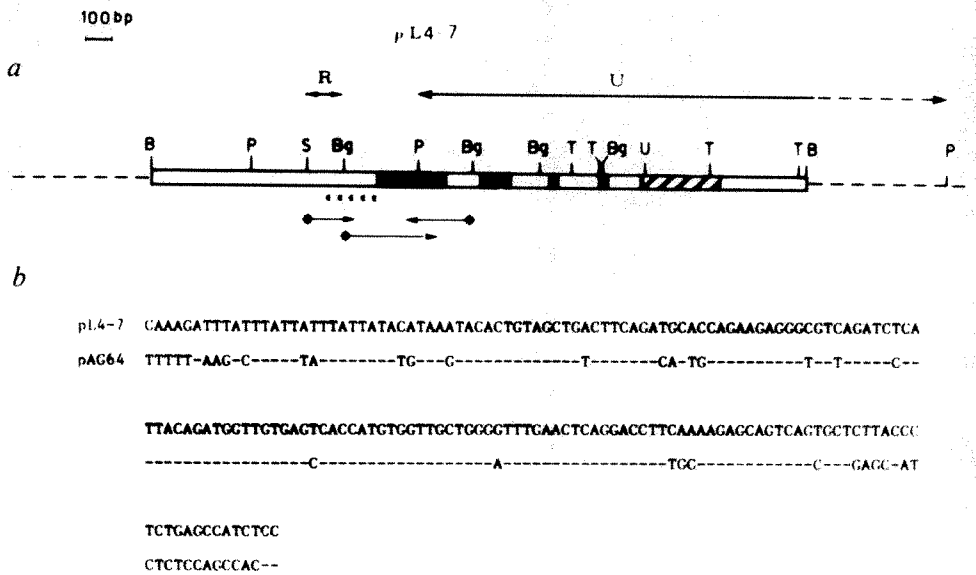


Fig. 1 *a*, Map of pL4-7. This plasmid was isolated originally by A. M. Frischauf, (EMBL) from DBA/2 mouse DNA. Fine restriction mapping and partial DNA sequencing have revealed that the pL4-7 is similar to the 3'-end portion of the 27.1 clone isolated by Steinmetz *et al.*[7]. Black boxes indicate the positions of the exons, a hatched box denotes the 3'-untranslated region as indicated by Steinmetz *et al.*[7]. Partial DNA sequencing was performed across exon 4 and we found no difference from the reported sequence of the 27.1 clone. The arrows indicate the direction and length of the sequences obtained by the chemical degradation method[27]. « « « Indicates the position of an *Alu*-like type II repeat. Subclone 'R' was constructed by inserting the 137-bp *Sal*I/*Bgl*II fragment in the *Bam*HI/*Sal*I sites of plasmid pAT153 (ref. 28). The *Sal*I/*Bgl*II fragment comprises 72 bp of the repetitive element and 65 bp of the adjacent intron sequences as determined by DNA sequencing. Subclone 'U', containing the 3'-end coding region devoid of the repetitive element, was constructed by subcloning the 2.7-kb *Pst*I fragment in the *Pst*I site of pAT153. B, *Bam*HI; Bg, *Bgl*II; P, *Pst*I; S, *Sal*I; T, *Sst*I; U, *Pvu*II. *b*, Comparison of the *Alu*-like repeat present in the pL4-7 and the repetitive element in the plasmid pAG64[1] written in the opposite orientation. -, Identical sequences.

Rat-1. No difference was detected between the Rat-1 and Py-rat cell DNAs (data not shown).

The presence of a type II *Alu*-like repeat within pL4-7 (Fig. 1) raises the question of the relationship between the mRNAs found in the Py-rat and the repetitive element. To answer this question, we have constructed subclones of pL4-7 containing either the repetitive element (subclone 'R'; see Fig. 1) or exclusively the non-repetitive *H-2* sequence (subclone 'U'; see Fig. 1), and have used these as probes in Northern transfer experiments. Figure 3 shows that probe R detects all the RNA transcripts observed with the pL4-7, whereas probe U detects only the 1.6-kb mRNA. These data indicate that the heterogeneous RNA transcripts of 0.5–0.8 kb hybridize only to *Alu*-like repeat, whereas the 1.6-kb mRNA is composed of the *RT.1* class I sequence containing an *Alu*-like type II repeat.

The activation of a MHC class I gene containing a repetitive element has been documented by Rigby's group[1,11,12] in SV40-transformed mouse cells and in two Py-mouse cell lines, where they have characterized sets of genes silent in most normal cells but active in oncogenic transformed mouse cells. The prototype complementary DNA clone of Set 1, pAG64 (ref. 1), encodes a *H-2* class I antigen and contains a dispersed repetitive element in the 3′-untranslated region of the mRNA which is very similar to the repetitive element located in the opposite orientation within one of the introns in pL4-7 (see Fig. 1*b*). Both these *Alu*-like elements are members of the *B2* family originally defined by hybridization to 'fold-back' heterogeneous nuclear RNA[13,14]. The *Alu*-like repeat in pL4-7 diverges 14% from the pAG64 repeat and 12% from the consensus *B2* family sequence[13].

Alu-like type II repetitive elements have been found in the 3′-untranslated region of cDNA clones encoded in the *H-2D* and *H-2L* regions[15–19], whereas cDNA clones encoding the K^b (ref. 20) and K^d (ref. 21) antigens lack repetitive sequences. The presence of *Alu*-like repeats in both mouse and rat MHC class I mRNAs may reflect a similar evolutionary history of their MHC.

Several conclusions can be drawn from our work. First, the elevated level of 1.6-kb mRNA appears to be independent of the expression of Py early large T-antigen [relative molecular mass (M_r) 100,000 (100K)] and small T-antigen (M_r 22K) (Table 1, Fig. 2), indicating that the presence of the 55K middle T-antigen is sufficient to induce a high level of 1.6-kb mRNA. As the 55K middle T-antigen is a non-nuclear protein and as there is no evidence that it can regulate gene transcription directly, one must postulate the presence of cell-encoded factor(s) involved in the activation of MHC class I mRNA in response to viral transformation. Second, the activation of 1.6-kb mRNA appears to be a reversible phenomenon, as low levels of 1.6-kb mRNA are found in the revertant cell line R3-rat. Third, elevated levels of 1.6-kb mRNA are found both in *in vitro* Py-rat cells and in cell lines established from tumours induced with Py-rat cells. Fourth, it appears that in neoplastic mouse and rat cells transformed by two similar oncogenic viruses, SV40 and Py, the activation of MHC class I mRNA containing a similar repetitive

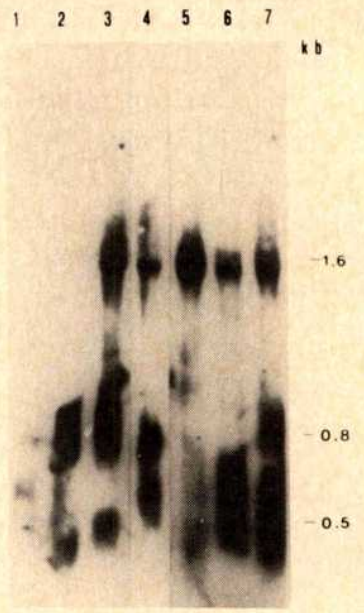


Fig. 2 RNA transfer hybridization analysis of normal and transformed rat cell lines. Polyadenylated cytoplasmic RNA was isolated as described previously[29]. Aliquots (2 μg) of poly(A)$^+$ RNA (selected once) were fractionated by electrophoresis in 1.3% agarose/formaldehyde gels and transferred to nitrocellulose filters[30]. The blots were heated for 2 h at 80 °C and prehybridized for 5 h at 42 °C in a buffer containing 5× Denhardt's solution (0.1% each of Ficoll, bovine serum albumin and polyvinylpyrrolidone), 0.25 mg denatured calf thymus DNA per ml and 50% formamide. Subsequently, the filters were hybridized at 42 °C in the same buffer containing 20 ng ml^{-1} ^{32}P-labelled pL4-7 DNA. The pL4-7 DNA was labelled by nick-translation[31] at a specific activity of 2×10^8 c.p.m. μg^{-1}. After hybridization, the filters were washed three times in 2×SSC, 0.1% SDS at room temperature, then at 50 °C for ~2 h. The filters were then autoradiographed at −80 °C with intensifying screen for 72 h. RNA samples are: lane 1, Rat-1; lane 2, R3-rat; lane 3, 7ax; lane 4, 7axT; lane 5, 82-rat; lane 6, Ag4-rat; lane 7, MT-rat. The gels were routinely calibrated with ribosomal RNA of various sources and with ^{32}P-labelled DNA plasmid size markers (×10^{-3}).

Table 1 Properties of the polyoma-transformed rat cell lines used

Cell lines	Ref.	T-antigen species				Isolation		
		Large-T	Middle-T	Small-T	Others	Foci	Agar	Tumour
7ax	22	+	+	+	−	+		
82-rat	22	−	+	+	63K, 40K, 32K	+		
53-rat	22	−	+	+	−	+		
H-2	23	+	+	+	−	+		
Ag4-rat		−	+	+	70K		+	
MT-rat		−	+	−	−	+		
7axT	8	−	+	+	75K			+
7axB	24	+	+	+	−			+
8aT	25	−	+	+	35K			+
82T	25	−	+	+	63, 40K, 32K			+
53T	25	−	+	+	−			+

The cell line MT-rat was isolated and characterized after DNA transfection of plasmid pPY-MT[26] on Rat-1 cells. The MT-rat cells contain about three copies of integrated Py DNA sequences, and express only the 55K middle T-antigen, as determined by immunoprecipitation with anti-T serum (L.L., unpublished results). The Ag4-rat cell line was isolated using a polyoma mutant Py9-1 which has lost part of the polyoma DNA sequences unique to the 100K large T-antigen coding sequences, but retains the ability to express middle and small T-antigens. The Ag4-rat cells express the 22K, 55K and 70K T-antigens, as determined by immunoprecipitation with anti-T serum (G.L.M. and L.L., unpublished results).

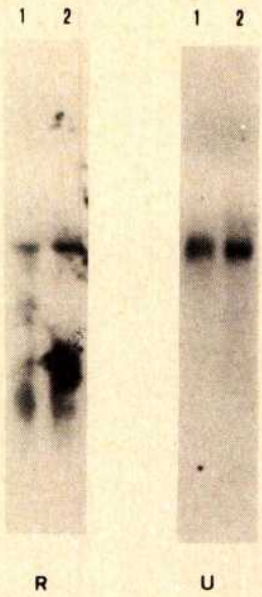


Fig. 3 Northern hybridization analysis of activated transcripts using the 'R' and 'U' probes (Fig. 1). Cytoplasmic poly(A)$^+$ RNA extracted from 7ax cells (lane 1) and 7axT (lane 2) were fractionated in 1.3% agarose/formaldehyde gels, then processed as described in Fig. 2 legend. Aliquots (5 μg) of poly(A)$^+$ RNAs were used. The exposure time was 1 week at −80 °C for blot 'R' and 48 h for blot 'U'.

element takes place. However, until the mRNA activated in the Py-transformed rat cells has been cloned, the homologous MHC class I genes activated in the mouse and rat transformed cells remain undefined.

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1. Brickell, P. M., Latchaman, D. S., Murphy, D., Willinson, K. & Rigby, P. W. J. *Nature* **306**, 756-760 (1983).
2. Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A. & van der Eb, A. *Nature* **305**, 771-775 (1983).
3. Gill, T. J., Cramer, D. V. & Kunz, H. W. *Am. J. Path.* **90**, 735-778 (1978).
4. Gunther, E. & Stark, O. *The Organization of the Major Histocompatibility Systems in Man and Animals* (ed. Gotze, D.) 207-253 (Springer, New York, 1977).
5. Kunz, H. W., Gill, T. J. & Mistra, D. N. *J. Immun.* **128**, 402-408 (1982).
6. Stock, W. & Gunther, E. *J. Immun.* **128**, 1923-1928 (1982).
7. Steinmetz, M. *et al. Cell* **25**, 683-692 (1981).
8. Lania, L., Hayday, A., Bjursell, G., Gandini-Attardi, D. & Fried, M. *Cold Spring Harb. Symp. quant. Biol.* **44**, 597-603 (1980).
9. Bernards, R. *et al. Nature* **305**, 776-779 (1983).
10. Southern, E. *J. molec. Biol.* **98**, 503-515 (1975).
11. Scott, M. R. D., Westphal, K-H. & Rigby, P. W. J. *Cell* **34**, 557-567 (1983).
12. Murphy, D., Brickell, P. M., Latchman, D. S., Willinson, K. & Rigby, P. W. J. *Cell* **35**, 865-871 (1983).
13. Krayer, A. S. *et al. Nucleic Acids Res.* **10**, 7461-7475 (1982).
14. Kramerov, D. A., Lekakh, I. V., Samarina, O. P. & Ryskov, A. P. *Nucleic Acids Res.* **10**, 7477-7491 (1982).
15. Hood, L., Steinmetz, M. & Malissen, B. *A. Rev. Immun.* **1**, 568-594 (1983).
16. Steinmetz, M. *et al. Cell* **24**, 125-134 (1981).
17. Lalanne, J. L. *et al. Nucleic Acids Res.* **10**, 1039-1049 (1982).
18. Reyes, A. A., Schold, M., Itakura, K. & Wallace, R. B. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3270-3274 (1982).
19. Moore, K. W., Sher, B. T., Sun, Y. H., Eakle, K. & Hood, L. *Science* **215**, 679-682 (1982).
20. Reyes, A. A., Schold, M. & Wallace, R. B. *Immunogenetics* **16**, 1-9 (1982).
21. Xin, J. H., Kvist, S. & Dobberstein, B. *EMBO J.* **1**, 467-471 (1982).
22. Lania, L. *et al. Virology* **101**, 217-232 (1980).
23. Fried, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2117-2121 (1983).
24. Hayday, A., Ruley, H. E. & Fried, M. *J. Virol.* **44**, 67-77 (1982).
25. Lania, L., Hayday, A. & Fried, M. *J. Virol.* **39**, 422-431 (1981).
26. Treisman, R., Novak, U., Favaloro, J. & Kamen, R. *Nature* **292**, 595-600 (1981).
27. Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
28. Twigg, A. J. & Sherratt, D. *Nature* **283**, 216-218 (1980).
29. Favaloro, J., Treisman, R. & Kamen, R. *Meth. Enzym.* **65**, 718-749 (1980).
30. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
31. Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-252 (1977).

Keratinocytes blocked in phorbol ester-responsive early stage of terminal differentiation by sarcoma viruses

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It has been suggested that the initiation step in mouse skin carcinogenesis involves an alteration in epidermal differentiation, as mouse basal keratinocytes exposed to initiators resist the arrest of cell growth that is normally associated with the induction of terminal differentiation by calcium ions[1-3]. The growth of epidermal basal cells infected by Kirsten (Ki) or Harvey (Ha) sarcoma viruses is, however, arrested in response to calcium ions, although the cells do not progress through their entire maturation programme when a functioning *ras* gene of those viruses is expressed[4]. If continuous proliferation in the differentiating cell layers is a requirement for tumour formation in skin[3,5], the response of sarcoma virus-infected cells seems inconsistent with the suggestion that an activated *ras* gene is sufficient to initiate skin carcinogenesis[6]. We now show that sarcoma virus-infected keratinocytes, when induced to differentiate, are blocked at an early, reversible stage of maturation. Furthermore, the cells respond to phorbol ester tumour promoters by undergoing a phenotypic reversion to a less mature stage. These results suggest that activation of a *ras* gene can produce conditionally initiated cells, in which the full expression of tumorigenicity depends on exposure to tumour promoters.

Mouse basal keratinocytes can be grown selectively in <0.1 mM Ca^{2+} medium (low Ca^{2+}) and induced to stop growing, differentiate terminally and slough from the culture dish by >0.1 mM Ca^{2+} medium (high Ca^{2+})[7]. (By previously published criteria for biological responses, any Ca^{2+} concentration <0.1 mM is considered low Ca^{2+} and >0.1 mM is considered high Ca^{2+}.) Carcinogen treatment of basal cells produces foci that continue to proliferate when challenged with high Ca^{2+} medium. Such studies have formed the basis for the hypothesis that this cellular change represents the initiation event[1-3,8]. It seems likely that cells that have undergone the initiation step possess all the information required to produce papillomas[3,5]. Recently, Balmain *et al.* have reported that Ha-*ras* expression is increased in epidermal papillomas and carcinomas[6] and that more than 60% of papillomas contain an activated *ras* gene when tested by 3T3 transfection studies[6,9]. However, we have been unable to detect elevated levels of *ras* messenger RNA or other mRNAs hybridizable by complementary DNAs for viral oncogenes in putatively initiated epidermal cell lines[29]. Furthermore, studies with sarcoma viruses indicate that an activated *ras* gene produces changes in keratinocytes which differ from those seen in putative chemically initiated cells and which are inconsistent with the expected requirements for papilloma formation[3,4]. To clarify this discrepancy, we made further studies of the properties of keratinocytes expressing an activated Ha-*ras* and Ki-*ras* gene that had been introduced by virus infection.

Two epidermal marker proteins were examined, the pemphigoid and pemphigus vulgaris antigens. Pemphigoid antigen is a protein of relative molecular mass (M_r) 220,000 (220K) found in the basement membrane and it is closely associated with the plasma membrane of the basal cell[10,11]. Pemphigus vulgaris antigen is a 210K glycoprotein containing disulphide-linked chains of 130K and 80K which occurs on the surface of stratifying epithelial cells[12]. Immunofluorescence and immunoprecipitation data have shown that the pemphigoid antigen is synthesized exclusively by basal cells in low Ca^{2+}; within 6 h of switch to high Ca^{2+}, pemphigoid synthesis is

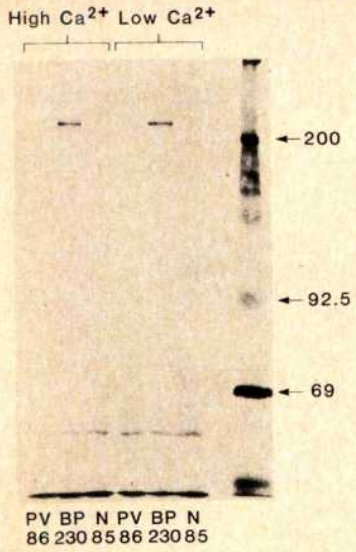


Fig. 1 Synthesis of epidermal antigens by virus-infected and control cells. Approximately 3×10^6 c.p.m. of each sample was immunoprecipitated with specific antisera (N, normal human serum; PV, pemphigus vulgaris antiserum no. 86; BP, bullous pemphigoid antiserum no. 230) and precipitates separated on a SDS-dithiothreitol (DTT) 6% polyacrylamide gel as reported previously[12]. Fluorograms were developed after 3 weeks of exposure. Numbers refer to M_r markers ($\times 10^{-3}$). Only virus-infected cultures are shown.

Methods. Newborn BALB/c mouse keratinocytes were isolated and cultured in Eagle's medium with 8% Chelex-treated fetal calf serum (Reheis), 1% antibiotic/antimycotic mixture (Gibco) and 0.05 mM Ca^{2+} as described previously[7]. On day 3 *in vitro*, cultures were exposed to a virus complex containing Friend murine leukaemia virus (F-MuLV) clone 57 and Kirsten sarcoma virus in the presence of 4 μg ml^{-1} polybrene as reported previously[4]. Viral stocks were titred by xc plaque formation in NIH 3T3 cells[4]. Cells were exposed to 1 : 2 dilution of virus stock (multiplicity of infection of ~0.5) for 1 h and a dilution eightfold further was continued for 72 h. Fresh medium (0.05 mM Ca^{2+}) was added for an additional 24 h after which half of the dishes were switched to medium containing 1.2 mM Ca^{2+}. Cultures were maintained in either 1.2 mM or 0.05 mM Ca^{2+} for 48 h of which the final 16 h included 12.5 μCi ml^{-1} ^{14}C amino-acid mixture (NEN, 55 mCi per mol atom carbon). The cell layer was extracted with 3 ml of 0.5% Nonidet P-40 in Tris-buffered saline (10 mM Tris HCl, *p*H 7.4, 0.15 mM NaCl) with 1 mM phenylmethylsulphonyl fluoride. After centrifugation at 100,000*g* for 1 h, the supernatant was dialysed against 0.3% Nonidet P-40 in Tris-buffered saline.

inhibited by >90%[13]. Similar studies have indicated that pemphigus vulgaris antigen is synthesized exclusively by differentiating cells in high Ca^{2+} (ref. 13). Therefore, we prepared cell lysates from virus-infected keratinocytes that had been labelled with ^{14}C-amino acids for 16 h in low or high Ca^{2+} medium, and examined them by gel separation of immunoprecipitates using specific antisera to pemphigus vulgaris or pemphigoid antigens (Fig. 1). We found that, as occurs with normal cells, pemphigoid antigen was synthesized by infected keratinocytes in low Ca^{2+}, but unlike normal cells, synthesis was unchanged in infected cells maintained in high Ca^{2+} for 48 h. Furthermore, the extremely low levels of labelled pemphigus vulgaris antigen, expected in lysates from low Ca^{2+} cells, were also observed in lysates of virus-infected cells grown in high Ca^{2+} medium, culture conditions which stimulate pemphigus vulgaris synthesis in normal cells. Because the expression of these proteins is strongly linked to the differentiation state of keratinocytes[13], the results suggested that virus-infected cells in high Ca^{2+} maintain basal cell properties.

Additional support for an early differentiative block of virus-treated cells was obtained in experiments in which cells were returned to low Ca^{2+} growth conditions after a period in high Ca^{2+}. Previously, Hennings and Holbrook[14] had shown that

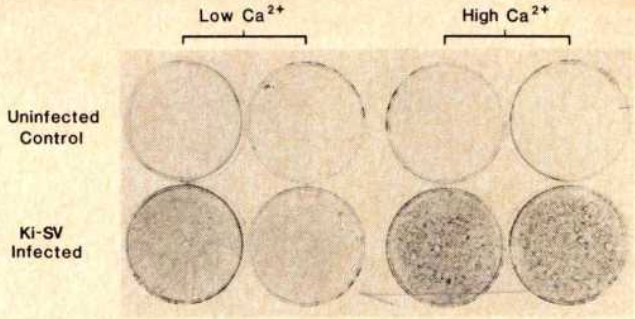


Fig. 2 Reversibility of the differentiation state of sarcoma virus-infected keratinocytes. Newborn BALB/c keratinocytes were isolated, plated and exposed to Kirsten virus as described in Fig. 1 legend. Five days after the start of virus exposure, all cultures were switched from 0.05 mM Ca^{2+} medium to 0.5 mM Ca^{2+} medium and maintained for 2 weeks with twice-weekly medium changes. Control and virus-infected dishes were then either switched back to 0.05 mM Ca^{2+} or continued in 0.5 mM Ca^{2+}, both for an additional 2 weeks. All dishes were then fixed in 10% formalin and stained with 10% Giemsa stain. Uninfected control cultures were exposed to polybrene only. Previously, controls exposed to polybrene only were shown to be identical to F-MuLV-exposed controls[4]. The low Ca^{2+} (0.05 mM) or high Ca^{2+} (0.5 mM) groups refer to the Ca^{2+} level of the final growth medium. Virus-treated cultures in 0.05 mM Ca^{2+} are not as dense as those in 0.5 mM Ca^{2+} and therefore do not stain as darkly, although both culture groups form completely confluent monolayers. Both uninfected control groups contained very few attached cells and thus show little staining.

keratinocytes are irreversibly committed to terminal differentiation by 72 h treatment in high Ca^{2+}. Such cells slough from culture even if switched back to the lower Ca^{2+}. This result is reproduced (Fig. 2) in uninfected control cultures, which were devoid of keratinocytes when maintained in high Ca^{2+} continuously for 4 weeks or when grown in high Ca^{2+} for 2 weeks and then returned to low Ca^{2+} for 2 weeks. The virus-infected cultures (Fig. 2) remained confluent when maintained in high Ca^{2+} conditions (although growth rate was low)[4]. When these cultures were returned to low Ca^{2+} medium, the confluent cell layer persisted and the cells assumed a basal cell morphology once again. These results support the suggestion that the sarcoma virus (and presumably the active *ras* gene) blocks keratinocytes prior to a step of irreversible commitment to terminal differentiation.

The state of epidermal differentiation is known to determine the response pattern to phorbol ester tumour promoters. Proliferative responses, such as the induction of ornithine decarboxylase (ODC), are characteristic of basal cells[15,16], and these responses are rapidly lost as cells begin their maturation programme[15]. Phorbol esters markedly accelerate terminal differentiation of more mature basal cells[16,17], as indicated by the induction of epidermal transglutaminase and an increase in the percentage of cells with cornified envelopes. Modulation of the normal pattern of phorbol ester responses would be expected if virus infection altered the maturation state of keratinocytes. Virus-infected basal cells incubated in low Ca^{2+} medium are highly sensitive to the induction of ornithine decarboxylase by 12-O-tetradecanoyl phorbol-13-acetate (TPA) (not shown). The response kinetics are similar to those of normal basal cells, although the magnitude is several-fold greater. When normal cells were induced to differentiate by Ca^{2+}, TPA was ineffective in inducing ODC within 6 h after shifting to high Ca^{2+} medium[15] (Fig. 3). However, virus-infected cells retained response to TPA for ODC induction even after several days in high Ca^{2+} medium (Fig. 3). These results are consistent with the suggestion that the virus blocks maturation at the basal cell stage.

Epidermal transglutaminase activity increases during Ca^{2+}-induced differentiation in normal cells[18] and virus infection does not block this increase[4]. We found that in normal cells, transglutaminase activity remained high as the cells completed their

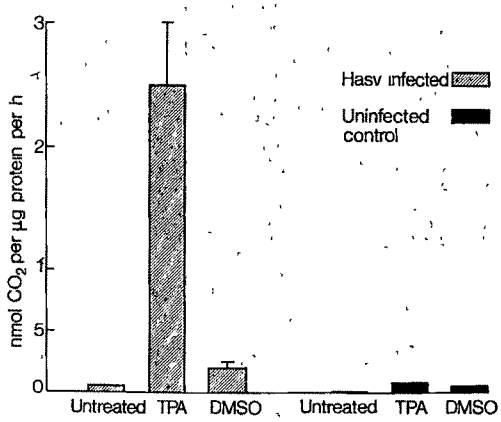


Fig. 3 Ornithine decarboxylase (ODC) response of keratinocytes in 1.2 mM Ca^{2+} medium after exposure to TPA. The viral preparation contained Harvey sarcoma virus with F-MuLV in this experiment, but identical results were obtained with cultures infected with Kirsten sarcoma virus.

Methods. Keratinocytes were plated and exposed to virus as indicated in Fig. 1. Cultures were exposed to virus or polybrene for 72 h in 0.05 mM Ca^{2+} medium and fresh 0.05 mM Ca^{2+} medium for 48 h and then switched to 1.2 mM Ca^{2+} medium for 72 h. Untreated dishes were washed three times with normal saline and frozen on dry ice, while treated cells received fresh 1.2 mM Ca^{2+} medium containing either 0.1% DMSO (Pierce) or 0.1 μg ml^{-1} TPA (Chemical Carcinogenesis, Eden Prairy, Minnesota) and 0.1% DMSO After a 6-h incubation at 37 °C, cells were washed and frozen as above Cells from each dish were scraped twice with 120 μl PBS and 50-μl aliquots of the lysates were assayed for ODC and protein as described previously[15].

Table 1 DNA synthesis in epidermal cells after exposure to TPA

Treatment	Control cells (0.5 mM Ca^{2+}) DNA synthesis (c.p.m. per μg DNA)	Harvey sarcoma virus-infected cells (0.5 mM Ca^{2+}) DNA synthesis (c.p.m. per μg DNA)
0.1% DMSO	511 ± 59	489 ± 73
0.0001 μg ml^{-1} TPA	260 ± 18	616 ± 27
0.001 μg ml^{-1} TPA	264 ± 8	665 ± 52
0.01 μg ml^{-1} TPA	299 ± 12	953 ± 175

Virus-infected or control cells were exposed to the test agent while in 0.5 mM Ca^{2+} medium. After 5 days, cultures were exposed to medium containing ^{3}H-thymidine. Values represent the mean ± s.e. of four samples for each exposure. DNA synthesis decreases in control cells exposed to TPA and increases in virus-infected cells.

Methods. Epidermal cells were isolated, cultured in 0.05 mM Ca^{2+} medium and infected with virus as described in Fig. 1 legend. Four days after infection, cultures were switched to 0.5 mM Ca^{2+} medium and continuously exposed to TPA or dimethyl sulphoxide (DMSO). After 5 days, the cultures were labelled with 1 μCi ml^{-1} [*Me*-^{3}H]thymidine (6.7 Ci $mmol^{-1}$, NEN) for 1 h, washed three times with phosphate-buffered saline (PBS) containing 1% unlabelled thymidine and frozen. Quantitation of label incorporated into DNA was performed as described previously[16].

differentiation programme, and this activity was not further influenced by TPA (Fig. 4), although initially TPA and Ca^{2+} acted synergistically to increase transglutaminase activity[17]. When virus-infected cells in high Ca^{2+} were exposed to TPA, there was a gradual decrease in transglutaminase activity; in some experiments, activity returned to that seen in low Ca^{2+}. Concurrent with the decrease in transglutaminase activity, TPA also caused a dose-dependent increase in ^{3}H-thymidine incorporation into DNA of virus-infected but not control cells in high Ca^{2+} (Table 1). These results suggest that maturing keratinocytes expressing an activated *ras* gene respond to phorbol ester tumour promoters by reverting to a less mature basal cell phenotype.

This new information on the biological state of keratinocytes infected with murine sarcoma viruses provides a biological

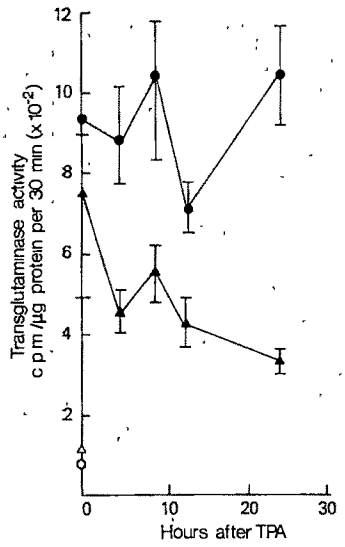


Fig. 4 TPA decreases epidermal transglutaminase in virus-infected cells. ●, Uninfected controls, 1.2 mM Ca^{2+}; ▲, Kirsten virus-infected, 1.2 mM Ca^{2+}; ○, uninfected control, 0.05 mM Ca^{2+}, △, Kirsten virus-infected, 0.05 mM Ca^{2+}. Values represent the mean ± range of duplicate determinations of duplicate samples. Identical results were obtained with Harvey sarcoma virus-infected cells

Methods. Keratinocytes were plated and infected with a 1:3 dilution of Kirsten sarcoma virus as in Fig. 1. After 72 h of virus exposure and 24 h of fresh 0.05 mM Ca^{2+} medium, dishes were switched to 1.2 mM Ca^{2+} medium for 48 h. Each group was treated with 0.1 μg ml^{-1} TPA and duplicate samples were washed and frozen on dry ice at 0, 4, 8, 12 and 24 h. Cells were lysed by 5 min incubation at 37 °C with PBS containing 1% Triton X-100 and 10 mM DTT. Transglutaminase was assayed on 50-μl aliquots of the lysate by adding 150 μl of the following freshly prepared mixture: 20 μl of 500 mM sodium borate, *p*H 9 5, 10 μl of 100 mM $CaCl_2$, 10 μl of 10 mM EDTA, 20 μl of casein (20 mg ml^{-1}, Sigma), 10 μl of [2,3-^{3}H]putrescine (NEN, 31.3 Ci $mmol^{-1}$) and 80 μl distilled water Samples and blanks (containing buffer used for scraping cells instead of cell lysate) were incubated for 30 min at 37 °C and 80-μl aliquots were spotted on Whatman 3MM disks for counting. Protein was measured by the method of Lowry after destroying DTT in 20-μl samples by addition of 250 μl of PBS containing 100 μg chloramine-T and incubation at room temperature for at least 30 min[28]

framework to explain how activation of a *ras* gene could yield a phenotype consistent with that hypothesized for initiated cells and required for epidermal tumorigenesis. Although these studies are confined to the effects of v-*ras*, both v-Ki-*ras* and v-Ha-*ras* produce similar effects. Thus, the results are probably relevant to other activated forms of the *ras* gene. We propose that an activated *ras* gene in keratinocytes provides the proliferative signal required to form a tumour, but that the cells are unable to maintain vertical growth above the basement membrane to produce a visible lesion. These cells may be indistinguishable from committed basal cells residing on the basement membrane, which lack proliferative potential[19]. Because virus-altered cells fail to complete their differentiation programme, they might be expected to accumulate within the epidermis and expand to a limited extent in the space available[19]. Exposure to tumour promoters would selectively reverse the quiescent growth state of those cells with an activated *ras* gene while normal committed basal cells would be unaffected or stimulated to differentiate further[14]. Repeated exposure to promoter, such as that required for tumour promotion, would maintain the permissive environment, allowing reactivated cells to grow in the suprabasal regions of the epidermis. Proliferation in the differentiating cell zone is a distinguishing feature of epidermal papillomas[3].

In many mouse strains, at least two classes of skin papillomas develop in protocols involving initiation and promotion[20]. Promoter-dependent tumours require repeated exposure to promoter for both expression and maintenance and regress on

withdrawal of the promoting stimulus[20]. Autonomous papillomas, once formed, do not require promoter exposure for maintenance and are more likely to progress to carcinomas[20,21]. The biological changes we have defined for keratinocytes expressing an activated *ras* gene are most consistent with *ras* activation being involved in the formation of promoter-dependent tumours. In contrast, the characteristics of chemically altered cells, selected by virtue of their resistance to differentiation, are most consistent with changes in autonomous papillomas. Whether the latter cell type requires more than one genetic change (of which *ras* activation could be a component and thus explain the isolation of an activated *ras* from 60% of papillomas) or an alteration in a different genetic element remains to be determined.

Both chemically altered and virus-altered cells fail to produce epidermal carcinomas in nude mice (ref. 22 and our unpublished data). Thus *ras* activation alone is not sufficient to complete the cancer process. We have shown previously that at least two genetic events are required to produce carcinomas in mouse skin[23]. Other studies have suggested that several viral oncogenes must work in concert to transform primary cultures of murine cells[24–26]. Weissman and Aaronson have shown that murine sarcoma viruses can elicit the transformed phenotype in aneuploid mouse epidermal cells[27]. Thus, the combination of an activated *ras* gene and aneuploidy may satisfy additional requirements for transformation.

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1 Kulesz-Martin, M, Koehler, B, Hennings, H & Yuspa, S H *Carcinogenesis* **1**, 995–1006 (1980)
2 Yuspa, S H & Morgan, D L *Nature* **293**, 72–74 (1981)
3 Yuspa, S H in *Cellular Interactions by Environmental Tumor Promoters* (eds Fujiki, H, Hecker, E, Moore, R E, Sugimura, T & Weinstein, I B) 315–326 (Japan Scientific Societies Press, Tokyo)
4 Yuspa, S H, Vass, W & Scolnick, E *Cancer Res* **43**, 6021–6030 (1983)
5 Yuspa, S H, Hennings, H & Lichti, U *J supramolec. Struct Cell Biochem* **17**, 245–247 (1981)
6 Balmain, A, Ramsden, M, Bowden, G T & Smith, J *Nature* **307**, 658–660 (1984)
7 Hennings, H *et al Cell* **19**, 245–254 (1980)
8 Kulesz-Martin, M, Kilkenny, A E, Holbrook, K A, Digernes, V & Yuspa, S H *Carcinogenesis* **4**, 1367–1377 (1983)
9 Balmain, A & Pragnell, I B *Nature* **303**, 72–74 (1983)
10 Stanley, J R, Hawley-Nelson, P, Yuspa, S H, Shevach, E M & Katz, S I *Cell* **24**, 897–903 (1981)
11 Stanley, J R, Woodley, D T & Katz, S I *J invest Derm* **82**, 108–111 (1984)
12 Stanley, J R, Koulu, C & Thivolet, C *J clin Invest* **74**, 313–320 (1984)
13 Stanley, J R & Yuspa, S H *J Cell Biol* **96**, 1809–1814 (1983)
14 Hennings, H & Holbrook, K in *Ions, Cell Proliferation and Cancer* (eds Boynton, A L, McKeehan, W L & Whitfield, J F) 499–516 (Academic Press, New York, 1982)
15 Lichti, U, Patterson, E, Hennings, H & Yuspa, S H *J cell Physiol* **107**, 261–270 (1981)
16 Yuspa, S H, Ben, T, Hennings, H & Lichti, U *Cancer Res* **42**, 2344–2349 (1982)
17 Yuspa, S H, Ben, T & Hennings, H *Carcinogenesis* **4**, 1413–1418 (1983)
18 Hennings, H, Steinert, P & Buxman, M M *Biochem biophys Res Commun* **102**, 739–745 (1981)
19 Potten, C S *Int Rev Cytol* **69**, 271–318 (1981)
20 Burns, F J, Vanderlaan, M, Sivak, A & Albert, R E *Cancer Res* **36**, 1422–1427 (1976)
21 Scribner, J D, Scribner, N K, McKnight, B & Mottet, N K *Cancer Res* **43**, 2034–2041 (1983)
22 Kilkenny, A E, Morgan, D, Spangler, E F & Yuspa, S H *Cancer Res* (in the press)
23 Hennings, H *et al Nature* **304**, 67–69 (1983).
24 Newbold, R F & Overell, R W *Nature* **304**, 648–651 (1983)
25 Land, H, Parada, L F & Weinberg, R A *Nature* **304**, 596–602 (1983)
26 Ruley, E H *Nature* **304**, 602–606 (1983)
27 Weissman, B E & Aaronson, S A *Cell* **32**, 599–606 (1983)
28 Higuche, M & Yoshida, F *Analyt Biochem* **77**, 542–547 (1977)
29 Toftgard, R, Roop, D R & Yuspa, S H *Carcinogenesis* (in the press)

Cigarette smoke induces DNA single-strand breaks in human cells

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Epidemiological evidence suggests that smoking is a major cause of human lung cancer[1,2]. However, the mechanism by which cigarette smoke induces the cancer remains obscure, although in tobacco carcinogenesis, promotion and/or co-carcinogenesis may have crucial roles. The epidemiological data show that if an individual stops smoking, the risk of his contracting lung cancer increases no further. Moreover, laboratory experiments show that cigarette smoke condensate (CSC) exhibits co-carcinogenic and promoting activities in tumour production and malignant transformation[3–5]. Clastogenic action is thought to be intimately involved in tumour promotion[6–8], and it is therefore interesting that visible chromosome changes such as chromosome aberrations and sister chromatid exchanges are known to be caused by cigarette smoke[9–11]. However, there has been no previous direct demonstration that cigarette smoke can cause single-strand breaks (SSB) in DNA. Here we report that cigarette smoke induces considerable numbers of DNA SSB in cultured human cells, and that such strand breaks may be ascribed to active oxygen generated from cigarette smoke.

Alkali-labile sites and SSB in DNA were analysed by an alkaline elution technique[12] Smoke from a commercial filter-tipped cigarette was passed through 6 ml of phosphate-buffered saline (PBS), *p*H 7.4, for 5 min and used immediately as 'smoke-PBS' (ref. 13). Human lung carcinoma (A549) cells prelabelled with ^{14}C-thymidine (50 nCi per nmol per Petri dish) were treated with smoke-PBS at 20 °C for 1 h.

Figure 1*a* shows the alkaline elution profile of human cell DNA after treatment with 1 ml of smoke-PBS. The cells showed ~50% plating efficiency after treatment with this volume of smoke-PBS and, according to dye exclusion tests, 100% of the cells excluded trypan blue immediately after smoke-PBS treatment. The number of SSB obtained using different amounts of smoke-PBS (Fig. 1*b*) was calculated by external standardization (4.05 SSB per 10^{10} daltons of DNA were produced in cells irradiated at 4 °C with 150 rad of γ rays of ^{60}Co at 37 5 rad min^{-1}). The number of SSB varied almost fivefold between experiments, probably because of the fluctuating conditions for trapping smoke and a subtle difference in the quality of individual cigarettes. However, when the same smoke-PBS was used, consistent results were obtained

Table 1 Effect of scavengers on DNA strand breaks caused by cigarette smoke or by H_2O_2 in a cell system

Treatment	SSB per 10^{10} daltons	%SSB relative to standard
Expt 1		
Smoke-PBS	55 1±3.2	100
Smoke-PBS+SOD (100 μg)	35 4±3.1	64
Expt 2		
Smoke-PBS	21.1±0 7	100
Smoke-PBS+catalase (2 μg)	3 4±0	16
Smoke-PBS+inactivated catalase (2 μg)	22.1±4.2	104
Expt 3		
H_2O_2 (32 μM)	9 5±0.8	100
H_2O_2 (32 μM)+catalase (2 μg)	1.6±0 2	17
H_2O_2 (32 μM)+inactivated catalase (2 μg)	8.3±2.2	87

The reaction mixture contained 3×10^5 cells and 1 0 ml of smoke-PBS The specific activities of catalase (Sigma) and SOD (Sigma) were 20,000 U mg^{-1} and 3,000 U mg^{-1}, respectively Inactivated catalase was prepared as follows Catalase (2 mg) was incubated in 1 ml of 0 1 M NaOH at 70 °C for 30 min and neutralized with 0 1 M HCl. Each experiment was performed in duplicate Other conditions were the same as in Fig. 1

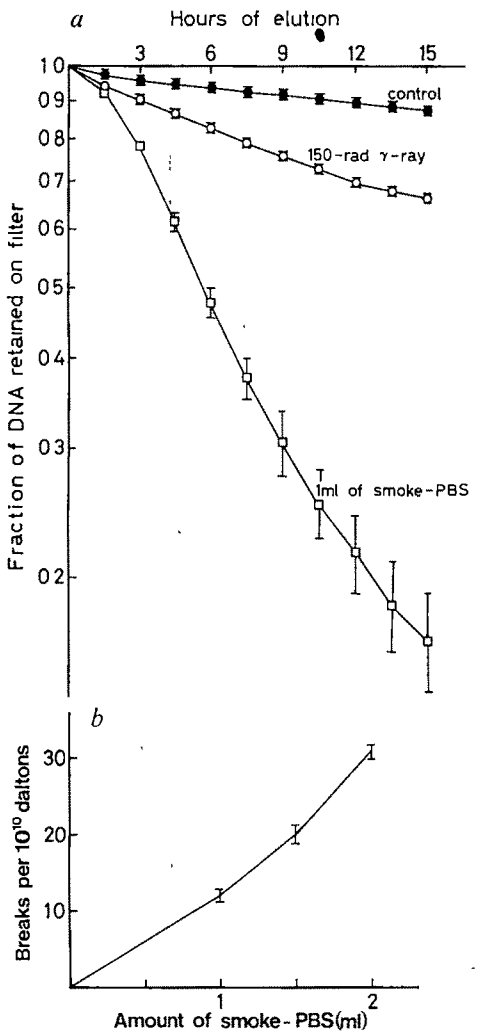


Fig. 1 *a*, Alkaline elution profiles of DNA from cells treated with 1 ml of PBS solution of cigarette smoke. *b*, Effects of different amounts of smoke-PBS on DNA strand breaks.
Methods. A549 cells (5×10^5 cells per dish) labelled with ^{14}C-thymidine were preincubated at 20 °C with 40 mM HEPES buffer (*p*H 7 2) containing heat-inactivated fetal calf serum at a final concentration of 7%. After 1 h incubation with (□) or without (●) smoke-PBS at 20 °C, the cells were washed twice with ice-cold PBS and collected with a rubber stripper in PBS on ice (As a standard for DNA strand breaks, cells were irradiated with 150 rad γ-rays from ^{60}Co and collected in the same way (○).) The cells were lysed on a polycarbonate filter (pore size 2.0 μm) with proteinase K (0.5 mg ml^{-1}) in the presence of 2% SDS, washed with 20 mM EDTA (*p*H 9.6) and eluted with 50 mM tetrapropylammonium hydroxide buffer containing 20 mM EDTA (*p*H 11 9) at a rate of 0.05 ml min^{-1}. Fractions were collected directly in scintillation vials at 90-min intervals. Radioactivity remaining on the filters was recovered by heating the filter at 70 °C for 1 h in 0.5 ml of 1 M HCl followed by the addition of 1 M NaOH All experiments were performed in duplicate and the bar shows two original points in each experiment

It was important to identify the component in cigarette smoke that is responsible for DNA SSB. Previously, we found that large amounts of active oxygen such as H_2O_2 and O_2^- were generated from cigarette smoke after trapping the smoke in PBS (~0 5 μg ml^{-1} of H_2O_2 was regularly detected in smoke-PBS)[13]. We also showed that SSB in DNA were produced by active oxygen radicals generated from active metabolites of carcinogenic aromatic amines[12]. These findings suggested that the active oxygen generated from cigarette smoke might be responsible for the observed SSB in DNA. To verify this, we checked the effect of catalase and superoxide dismutase (SOD) on the number of SSB produced, and found that both enzymes inhibited SSB, the inhibition with catalase being greater than with SOD (Table 1). Although the absolute number of SSB varied in several

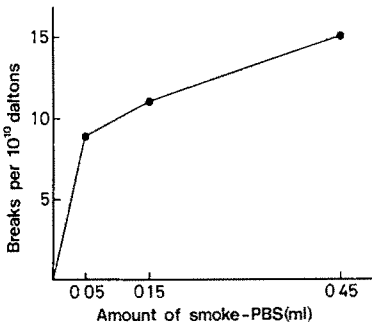


Fig. 2 Effect of different amounts of smoke-PBS on DNA strand breaks in a cell-free system. A549 cells (4×10^5 cells per dish) labelled with ^{14}C-thymidine were collected by trypsin treatment, put on polycarbonate filters and lysed with proteinase K (0.5 mg ml^{-1}) in the presence of 2% SDS The filters were washed twice with 20 mM EDTA (*p*H 9 6), then 5 ml PBS, 20 mM EDTA (*p*H 7 4) containing the indicated amount of smoke-PBS was added to the cell lysate on the filter with the outlet of the filter closed. The reaction mixture was kept in the dark at room temperature for 1 h, and the filters were then washed twice with 3 ml 20 mM EDTA (*p*H 9 8) and eluted with tetrapropylammonium hydroxide buffer (*p*H 11.9) containing 20 mM EDTA at a rate of 0.05 ml min^{-1} The subsequent treatment and calculation of SSB were the same as for the cell system (see Fig 1 legend)

Table 2 Effect of scavengers on DNA strand breaks caused by cigarette smoke in a cell-free system

Treatment	% SSB relative to standard
Standard	100
+SOD (10 μg)	11
+catalase (2 μg)	8
+inactivated catalase (2 μg)	100
+sodium benzoate (50 mM)	18
+DETAPAC (1 mM)	44

The standard system includes 5 ml of PBS (*p*H 7.4) containing 20 mM EDTA and 50 μl of smoke-PBS, and was incubated at room temperature for 1 h. Each value was obtained from four independent experiments performed in duplicate The average error was about 10% in each duplicated experiment. The SSB value of the standard was 6.2 ± 1.6 (mean ± s.d) SSB per 10^{10} daltons

independent experiments, the inhibition rates of the two enzymes showed a similar range (catalase, 85–95%; SOD, 30–45%). Inactivated catalase showed no effect in these inhibition experiments. Comparison of the activity of catalase and inactivated catalase in parallel, using authentic H_2O_2, showed clearly that the inhibitory effect of catalase was a result of its enzyme activity of quenching H_2O_2.

To exclude completely the contribution of DNA repair in SSB formation and to elucidate the mechanism of DNA scission more precisely, we looked for SSB in cell-free *in vitro* systems. Strand breaks were detected in protein-free DNA of high relative molecular mass which had been treated with smoke-PBS on polycarbonate filters, the number of SSB increasing dose dependently (Fig. 2). Table 2 shows the effect of several scavengers in a cell-free system. Catalase and SOD decreased the number of SSB, as did sodium benzoate, a hydroxyl radical (·OH) scavenger, while 1 mM diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DETAPAC), a potent Fe^{3+} chelator, inhibited SSB in the presence of 20 mM EDTA. In cigarette smoke, ·OH could be formed through the Fenton-type Haber–Weiss reactions and/or the reaction of H_2O_2 with nitrogen oxides[13,14].

$$H_2O_2 + O_2^- \xrightarrow{Fe^{3+}} O_2 + OH^- + {\cdot}OH$$
$$H_2O_2 + NO_x \longrightarrow {\cdot}OH + HONO_x$$

Accordingly, it is probable that the SSB observed were caused by OH that had been formed by these reactions, which in their turn had been induced by cigarette smoke. From these results, we conclude that cigarette smoke has clastogenic activity on cellular DNA to produce SSB as detected by alkaline elution, and that such activity can be explained mainly by the action of active oxygen generated from cigarette smoke.

Although mutagenicity induced by CSC has been found in bacterial and mammalian cells[9], this does not necessarily mean that the mutagenic compounds are directly involved in tobacco carcinogenesis[15]. On the other hand, the frequency of benzo(*a*) pyrene-induced transformation of Syrian hamster embryo cells is enhanced by CSC as efficiently as by the tumour promoter phorbol myristate acetate[4,5] These facts and the recent findings that the clastogenic action of active oxygen is involved in the promoting process suggest an important role for DNA SSB induced by cigarette smoke[6-8]. The active oxygen is generated mostly from polyphenols, such as catechol, its methyl derivatives and hydroquinone, which occur in abundance in the smoke[13]. This is consistent with the previous finding that the co-carcinogenic fraction in CSC contains catechol and hydroquinone and that catechol shows potent co-carcinogenic action in benzo(*a*)pyrene carcinogenesis[16]

A good correlation has been found between free radical formation, generation of active oxygen and carcinogenicity by aromatic amine derivatives and other carcinogens[17-23]. This may imply a causal significance of SSB in carcinogenesis, because the SSB in DNA were generated by active oxygen from active metabolites of naphthylamines[12]. In the present experiments, one cigarette produced ~10^4 SSB per cell. Although in the body, most SSB caused by cigarette smoke are expected to be efficiently repaired, some would remain unrepaired and the accumulation of such SSB over a long period may have serious consequences, especially for heavy smokers.

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1 Doll, R & Peto, R *Br med J* 2, 1525-1536 (1976)
2 Doll, R *Cancer Res* 38, 3573-3583 (1978)
3 Hecht, S S, Carmella, S, Mori, H & Hoffman, D *J natn Cancer Inst* 66, 163-169 (1981)
4 Rivedal, E & Sanner, T *Cancer Lett* 10, 193-198 (1980)
5 Rivedal, E, Hemstad, J & Sanner, T *Mechanisms of Toxicity and Hazard Evaluation* (eds Holmstedt, B, Lauwerys, R, Mercier, M & Roberfroid, M) 259-263 (Elsevier, Amsterdam, 1980)
6 Ement, I & Cerutti, P A *Proc. natn Acad Sci USA* 79, 7509-7513 (1980)
7 Birnboim, H C *Science* 215, 1247-1249 (1982)
8 Copeland, E S *Cancer Res* 43, 5631-5637 (1983)
9 DeMarini, D M *Mutat Res* 114, 59-89 (1983)
10 Hopkin, J M & Evans, H J *Nature* 279, 241-242 (1979)
11 Benedict, W F *et al Mutat Res* 136, 73-80 (1984)
12 Kaneko, M, Nakayama, T, Kodama, M & Nagata, C *Gann* 75, 349-354 (1984)
13 Nakayama, T, Kodama, M & Nagata, C *Gann* 75, 95-98 (1984)
14 Pryor, W A *Ann N Y Acad Sci* 393, 1-22 (1982)
15 Wynder, E L & Hoffman, D in *Tobacco and Tobacco Smoke, Studies in Experimental Carcinogenesis*, 225-231 (Academic, New York, 1967)
16 Van Duuren, B L & Goldschmidt, B M *J natn Cancer Inst* 56, 1237-1242 (1976)
17 Hozumi, M *Gann* 60, 83-90 (1969)
18 Kimura, T, Kodama, M & Nagata, C *Carcinogenesis* 3, 1393-1396 (1982)
19 Nakayama, T, Kimura, T, Kodama, M & Nagata, C *Carcinogenesis* 4, 765-769 (1983)
20 Stier, A, Clauss, R, Lucke, A & Reitz, I *Xenobiotica* 10, 661-673 (1980)
21 Floyd, R A, Soong, L M, Stuart, M A & Reigh, D L *Photochem Photobiol* 28, 857-862 (1978)
22 Nagata, C, Kodama, M, Ioki, Y & Kimura, T in *Free Radicals and Cancer* (ed Floyd, R A) 1-62 (Dekker, New York, 1982)
23 Lesko, S A, Lorentzen, R J & Ts'o, P O P in *Polycyclic Hydrocarbons and Cancer* Vol 1 (eds Gelboin, H V & Ts'o, P O P) 261-269 (Academic, New York, 1978)

Characterization of cDNA and genomic clones encoding the precursor to rat hypothalamic growth hormone-releasing factor

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Growth hormone-releasing factor (GHRF) is a hypothalamic peptide which positively regulates the synthesis and secretion of growth hormone in the anterior pituitary. The amino-acid sequence of a 43-residue GHRF peptide isolated from rat hypothalamus was recently determined[1]. Immunocytochemical techniques have been used to localize GHRF-containing cell bodies and nerve fibres largely to the medial-basal region of the rat hypothalamus[2]. The rat has also been used extensively as an animal model to study the effects of GHRF on growth hormone synthesis[3,4] and secretion[5,6] and on somatic growth[7]. To pursue questions concerning the biosynthesis of GHRF, the expression of the *ghrf* gene, and its regulation in the hypothalamus by neural and hormonal influences, we have now isolated and characterized both complementary DNA and genomic clones encoding rat hypothalamic GHRF. The rat *ghrf* gene spans nearly 10 kilobases (kb) of rat genomic DNA, contains 5 exons and encodes a 104-amino-acid precursor to the rat GHRF peptide. Comparison with previously characterized human *ghrf* cDNA[8,9] and genomic[10] clones has allowed patterns of conservation of amino-acid and nucleotide sequences between the human and rat GHRFs to be determined.

A 100,000-member cDNA library was constructed in the bacterial expression vector pUC8 (ref. 11) using as template poly(A)$^+$ RNA isolated from rat medial-basal hypothalamus, which was shown previously by immunohistochemical techniques to contain a concentration of GHRF-reactive cell bodies[2,12] This library was screened by hybridization using the insert from a human *ghrf* cDNA clone as probe[9], and two positive colonies were identified and isolated. Figure 1*a* shows the DNA sequence of the insert from one of these clones, prghrf-2 This cDNA includes a sequence encoding the complete 43-amino-acid rat GHRF peptide predicted from the amino-acid sequence data of Vale and co-workers[1] The structure of the entire rat *ghrf* cDNA insert is shown schematically in Fig 1*b* The GHRF peptide sequence is followed by a single arginine residue which presumably serves as a signal for proteolytic processing of GHRF from the precursor protein[13] This arginine is not preceded by a glycine residue, which is believed to serve as the amide donor in processed peptides that are C-terminally amidated[14]. This agrees with the published amino-acid sequence suggesting a free C-terminus[1], and makes rat GHRF different from human, bovine, ovine, porcine and caprine GHRFs, all of which are C-terminally amidated[15]. In cDNA clone prghrf-2, the GHRF peptide sequence is followed by a 30-amino-acid peptide of unknown function, a 105-nucleotide 3'-non-translated sequence and a poly(A) tract (Fig 1*b*) Comparison with previously characterized human *ghrf* cDNA clones[8,9] suggests that this rat *ghrf* cDNA clone lacks the N-terminal portion of the precursor, including the presumed signal peptide sequence and the 5'-non-translated sequences

The rat *ghrf* cDNA clone was used to screen a rat genomic library constructed in the λ vector Charon 4A (ref 16) Figure 2*a* shows the structures of two overlapping clones isolated from this library (rghrfλ101 and rghrfλ103) which predict the entire structure of the rat *ghrf* gene Hybridization identifies 5 exons which span more than 9 kb of rat genomic DNA, and reveals a structure very similar to that previously determined for the human *ghrf* gene[10]. Southern blot analysis of rat genomic DNA indicates that the rat *ghrf* gene is probably single-copy (data not shown). To define the structure of this gene more precisely, the DNA sequences of all exons, intron/exon boundaries and 5'- and 3'-flanking regions were determined by the strategy indicated (Fig 2*b*). Figure 3 gives the DNA sequence of the rat *ghrf* gene All four introns begin and end, respectively, with the consensus sequences GT and AG[17] Consensus TATA and CAT sequences, found in the 5'-flanking region of many eukaryotic genes[17,18], are located 31 and 76 nucleotides, respectively, 5' of the proposed messenger RNA cap site The location of the proposed mRNA cap site is based on its distance from the TATA

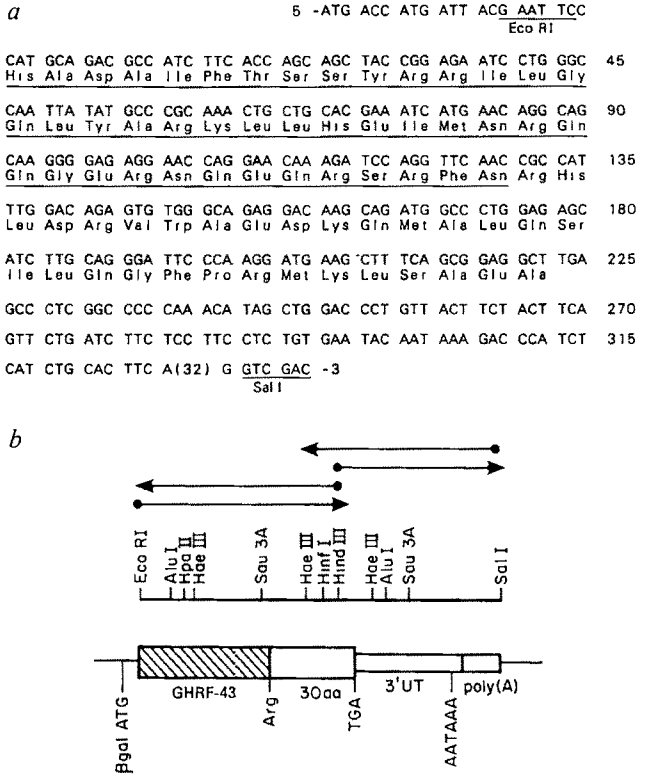


Fig. 1 Sequence and structure of a cDNA clone encoding rat hypothalamic growth hormone-releasing factor. *a*, DNA sequence of the insert from rat *ghrf* cDNA clone prghrf-2. The amino-acid sequence predicted from the open reading frame within the insert is shown beneath the DNA sequence. *Eco*RI and *Sal*I indicate the synthetic linkers ligated to the 5′ and 3′ ends of the cDNA insert, respectively. The sequence of the 43-amino-acid rat GHRF peptide is underlined *b*, Diagram of the structure of rat GHRF cDNA clone prghrf-2. The *ghrf*-coding region, the 30-amino-acid C-terminal peptide, the 3′-non-translated region and the poly(A) tract are indicated The positions of the *Escherichia coli* β-galactosidase initiation codon within the vector and the natural stop codon with the insert are also indicated. The restriction map indicates sites for common 4- and 6-bp cutters within the insert, and the arrows indicate the strategy used for DNA sequence analysis of the insert. **Methods.** Poly(A)$^+$ RNA was isolated using standard techniques from medial-basal hypothalamic fragments obtained from 150-g Zivic-Miller male rats that had been adrenalectomized for other purposes 3–4 days before they were killed. (These fragments were kindly provided by Dr Michael Browstein, NIH) This RNA was used as a template for oligo(dT)-primed first-strand cDNA synthesis with avian myeloblastosis virus reverse transcriptase. Second-strand cDNA synthesis was performed using the large fragment of DNA polymerase I, and *Sal*I and *Eco*RI linkers were added sequentially to the 3′ and 5′ ends of the double-stranded cDNA, respectively. Approximately 100 ng of cDNA were ligated into the bacterial expression vector pUC8 that had previously been digested with *Eco*RI and *Sal*I and the ligated mixture was used to transform $CaCl_2$-competent *E. coli* strain DH-1. Bacteria were plated at a density of 1,000 colonies per 82-mm plate onto L-broth agar with ampicillin, replica plated to nitrocellulose filters, and the filters screened by hybridization to the 350-bp *Eco*RI/*Bam*HI insert from human *ghrf* cDNA clone phghrf-54 (ref. 9), which had been labelled by nick-translation using [α-^{32}P]dCTP. Positive colonies from the high-density screen were re-screened at lower density until pure. Two positive colonies were isolated from 100,000 recombinants screened Because initial analysis indicated that both clones contained similarly sized inserts, only one was analysed in detail. Subsequent analysis indicated that the second cDNA clone contained a single additional G residue at the 5′ end of the insert. All cloning and screening procedures have been described in detail elsewhere[23]. DNA sequence analysis was by the chemical method of Maxam and Gilbert[24], using fragments that had been labelled using bacterial alkaline phosphatase, T4 polynucleotide kinase and [γ-^{32}P]ATP Enzymes were from either Bethesda Research Laboratories or New England Biolabs, and labelled nucleotides were from NEN

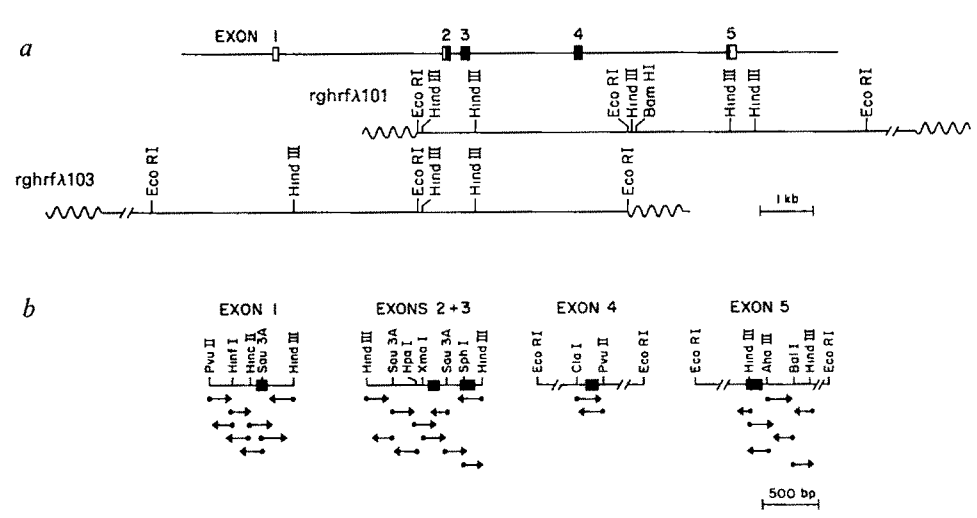


Fig. 2 Structure of rat growth hormone-releasing factor genomic clones and subclones. *a*, The top line indicates the deduced structure of the entire rat *ghrf* gene. Open boxes indicate non-coding exon regions and closed boxes indicate coding exon regions. Below this are indicated the structures of λ phage genomic clones rghrfλ101 and rghrfλ103. *Eco*RI, *Bam*HI and *Hin*dIII restriction enzyme sites used to establish overlap between the clones are indicated The wavy lines indicate the λ vector DNA, slashes indicate a break in the scale *b*, Regions of the above λ phage clones which were subcloned into plasmid vectors for further analysis are indicated, as is the strategy used to determine the DNA sequence of the rat *ghrf* gene Boxed regions are exons as indicated and selected restriction enzyme recognition sites used in the sequence analysis are shown Slashes represent a break in the scale. Dots represent sites that were labelled for DNA sequencing and the arrows indicate the direction and extent of derived sequence information **Methods.** Approximately 400,000 plaques from a rat genomic library consisting of partial *Eco*RI-digested rat DNA in the λ phage vector Charon 4A[16] were screened on nitrocellulose filters by hybridization to the 350-bp *Eco*RI/*Sal*I insert from rat *ghrf* cDNA clone prghrf-2 which had been labelled by nick-translation using [α-^{32}P]dCTP Two types of clones, represented by those shown in *a*, were isolated Exon regions were located by restriction mapping/DNA blotting techniques using appropriate probes. The rat cDNA clone was used as probe to identify exons 3, 4 and 5 Human *ghrf* cDNA clone phghrf-54 (ref. 9) was used to locate rat exon 2, and genomic probes isolated from the human *ghrf* gene[10] were used to localize rat exon 1 Regions from the genomic clones indicated in *a* were subcloned into plasmid pBR322 for DNA sequence analysis, which was by the chemical method of Maxam and Gilbert[24] Details of all cloning and mapping methods have been described previously[23]. Materials were from the sources indicated in Fig. 1 legend.

box[17,18], the fact that transcription generally begins at an A residue[19], and its homology to the cap site of the human *ghrf* gene, which has been determined by RNA mapping techniques[10]. The sequence AATAAA, thought to be important in polyadenylation[20], is located 21 nucleotides 5′ of the poly(A) addition site.

A combined analysis of the rat *ghrf* cDNA and genomic clones allows the entire amino-acid sequence of the rat GHRF precursor protein to be deduced. This sequence is compared with that of the human GHRF precursor protein in Fig. 4*a*. The GHRF peptides themselves are closely homologous, but differ substantially at their C-terminus (Fig. 4*a*) Because most of the

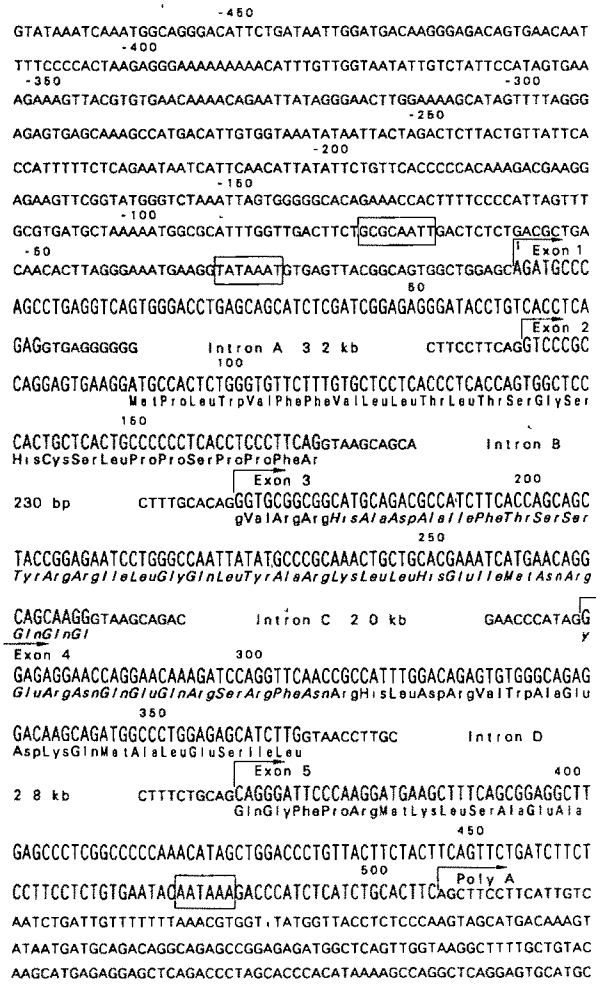


Fig. 3 DNA sequence of the rat growth hormone-releasing factor gene The DNA sequences of all exons, intron/exon boundaries and 5'- and 3'-flanking regions are shown. Numbering is from the proposed mRNA 5' cap site and the introns are not numbered. Arrows indicate the beginning of exons, or the poly(A) addition site (last arrow) The larger letters represent exons of the gene, exons were deduced either by identity with the rat *ghrf* cDNA or by homology with the exons of the human *ghrf* gene. The first and last 10 nucleotides of each intron are shown together with an approximate length of each intron The amino acids indicated in italic print are those of the 43-amino-acid GHRF peptide. The boxed nucleotides indicate consensus sequences as described in the text, they are the CAT box, TATA box and AATAAA sequence, in that order

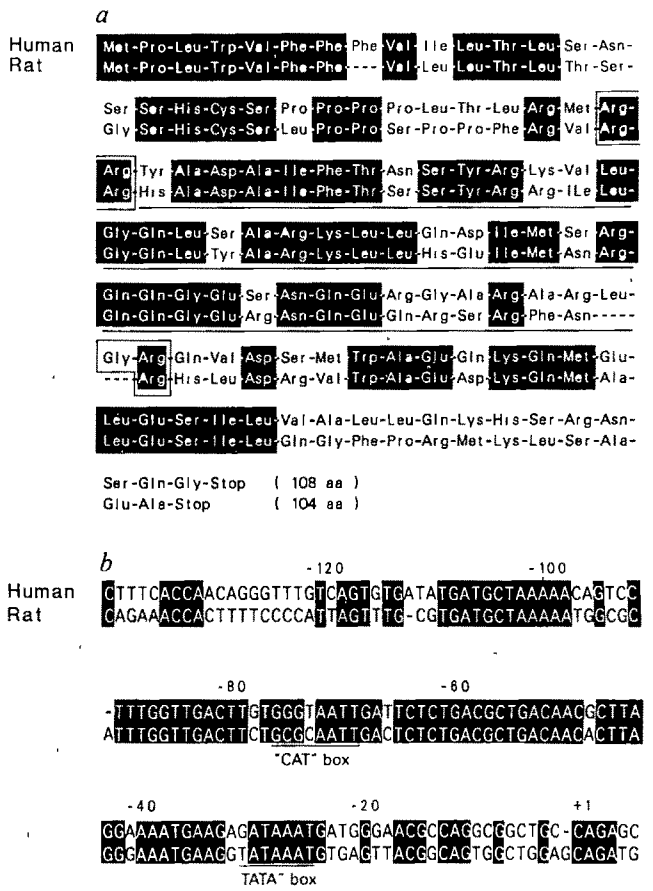


Fig. 4 Comparisons between the rat and human growth hormone-releasing factor precursor and gene sequences *a*, Comparison of the deduced amino-acid sequences of the complete rat and human GHRF precursor proteins. Identical residues are shaded; several gaps have been inserted to maximize the alignment. The GHRF peptide sequence is underlined and residues thought to be involved in the processing of this peptide from the precursor are boxed. *b*, Comparison of the nucleotide sequences in the 5'-flanking region of the human and rat *ghrf* genes Identical nucleotides are shaded, several gaps have been inserted to maximize the alignment. The positions of the consensus CAT and TATA box sequences are indicated.

biological activity of GHRF is localized to the N-terminal two-thirds of the peptide[21,22], changes in the C-terminal portion of the peptide can apparently be tolerated without loss of function. Proteolytic processing of GHRF from its precursor protein is expected to yield a C-terminal peptide of unknown function. Comparison of the human and rat precursor sequences (Fig. 4*a*) shows that the N-terminal half of this peptide is well conserved, whereas the C-terminal half diverges completely between rat and human. Analysis of the rat *ghrf* gene sequence (Fig. 3) reveals that this point of divergence corresponds precisely to a point where the precursor coding sequence is interrupted by intron D. Although the DNA sequence in this region is conserved between the human and rat *ghrf* genes, an apparent mutational event has resulted in a change in the 5' splice donor site used in the human versus the rat gene, shifting the translational reading frame (data not shown). Comparison of the human and rat *ghrf* gene DNA sequences indicates that although the two genes are homologous throughout, a particularly high degree of homology is found in their 5'-flanking regions (Fig. 4*b*). The sequence from −23 to −109 is 86% identical, compared with 76% identity between the *ghrf*-coding regions, 68% identity between the entire coding regions and ~58% identity between both the 5- and 3'-non-translated sequences. We presume that this high conservation of non-coding sequences in the 5'-flanking region reflects a functional importance, perhaps in the regulation of *ghrf* gene expression.

Because GHRF is believed to be an important physiological regulator of growth hormone synthesis and secretion[3-6], analysis of its synthesis and actions can be expected to lend insight into mechanisms by which the neural and hormonal control of somatic growth are integrated. The isolation of rat *ghrf* cDNA and genomic clones will enable us to use an animal model system to pursue questions concerning the expression of the *ghrf* gene in rat hypothalamus, to study its regulation by neural and hormonal influences, and to define the DNA sequences responsible for expression and regulation of this gene.

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1 Spiess, J , Rivier, J & Vale, W *Nature* **303**, 532-534 (1983)
2 Merchenthaler, I , Vigh, S , Schally, A & Petrusz, P *Endocrinology* **114**, 1082-1085 (1984)
3 Barinaga, M *et al Nature* **306**, 84-85 (1983)
4 Gick, G G *et al Proc natn Acad Sci USA* **81**, 1553-1555 (1984)

5. Wehrenberg, W. B. *et al. Biochem. biophys. Res. Commun.* **109**, 382-387 (1982).
6. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J. & Rivier, J. *Endocrinology* **112**, 1553-1555 (1983).
7. Wehrenberg, W. B., Bloch, B. & Phillips, B. J. *Endocrinology* **115**, 1218-1220 (1984).
8. Gubler, U. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4311-4314 (1983).
9. Mayo, K. E., Vale, W., Rivier, J., Rosenfeld, M. G. & Evans, R. M. *Nature* **306**, 86-88 (1983).
10. Mayo, K. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 63-67 (1985).
11. Vieira, J. & Messing, J. *Gene* **19**, 259-268 (1982).
12. Bloch, B. *et al. Nature* **301**, 607-608 (1983).
13. Steiner, D. F., Quinn, P. S., Chan, S. J., Marsh, J. & Tager, H. S. *Ann. N.Y. Acad. Sci.* **343**, 1-16 (1980).
14. Bradbury, A. F., Finnie, M. D. A. & Smyth, D. G. *Nature* **298**, 686-688 (1982).
15. Ling, N. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 4302-4306 (1984).
16. Sargent, T. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 3256-3260 (1979).
17. Breathnach, R. & Chambon, P. *A. Rev. Biochem.* **50**, 349-383 (1981).
18. Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. *Nucleic Acids Res.* **8**, 127-142 (1980).
19. Corden, J. *et al. Science* **209**, 1406-1414 (1980).
20. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211-214 (1976).
21. Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300**, 276-278 (1982).
22. Guilleman, R. *et al. Science* **218**, 585-587 (1982).
23. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
24. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).

Clusters of CpG dinucleotides implicated by nuclease hypersensitivity as control elements of housekeeping genes

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DNA sequences of the X-chromosome-linked hypoxanthine phosphoribosyltransferase (HPRT) and glucose 6-phosphate dehydrogenase (G6PD) genes have revealed the presence of clusters of CpG dinucleotides[1-3], raising the possibility that such clusters are involved in the control of expression of these genes, which are expressed in all tissues. Although CpG clusters are not exclusive features of the X chromosome[4-6], the analysis of X-linked genes provides the means to determine whether CpG clusters are control elements; one of the two homologous X loci in female mammals is not expressed[7], so that active and inactive versions of the gene can be compared. In fact, it has been shown that these CpG clusters are undermethylated when the gene is active and extensively methylated when the gene is inactive[2,8]. In addition to hypomethylation, chromatin hypersensitivity to endonuclease digestion is a known hallmark of regulatory sequences in eukaryotic genes[9]. We report here that the CpG clusters of the active *hprt* and *g6pd* genes are not only undermethylated, but also hypersensitive to *Msp*I, DNase I and S_1 nuclease, further supporting the suggestion that they are involved in the control of expression of these genes.

Figure 1*a* shows a restriction enzyme map of the 5′ region of the human *hprt* locus. A notable feature of the promoter region is a dense cluster of CpG dinucleotides (12 *Hpa*II and 15 *Hha*I sites within a 1.0-kilobase (kb) segment)[2]. As probe for studies of the *hprt* locus, we have used an intron sequence that hybridizes only to the *hprt* locus that is on the human X chromosome (pPB1.7)[1,2].

Figure 2*a* shows that the CpG cluster from the active *hprt* gene is extremely sensitive to digestion with *Msp*I, whereas the cluster from the inactive gene is not. When chromatin of male lymphoblasts (XY) is digested for 20 min with only 0.1 U of *Msp*I per μg DNA, the single active gene is completely cleaved, as shown by the presence of only the 9.5-kb sub-band in *Eco*RI digests of the purified DNA; this sub-band is also seen in chromatin digests from female lymphocytes. However, the 11.5-kb uncleaved fragment is also present, even if 10 times more *Msp*I is used. We infer that the uncleaved fragment comes from the inactive X because it is found exclusively in females and because the intensity of the band is proportional to the number of inactive X chromosomes (Fig. 2*a*, XX, XXX and XXXX lymphocytes). Further evidence that the inactive gene is resistant comes from studies of mouse/human hybrid cells, which contain the human inactive X homologue but not the active one. These hybrids are also a source of reactivants that have activated the *hprt* gene on the inactive X. Our observations of these reactivants indicate that acquisition of expression is accompanied by acquisition of nuclease hypersensitivity. When the gene is inactive (that is, on the isolated inactive X), the CpG cluster is resistant to *Msp*I digestion (Fig. 2*b*). However, when the *hprt* locus is re-expressed[2] as a result of reactivation of the locus on the inactive X, either spontaneously (X Rea-1) or induced by 5-azacytidine (X Rea-2), the cluster becomes as sensitive to *Msp*I as that on the active X (Fig. 2*b*).

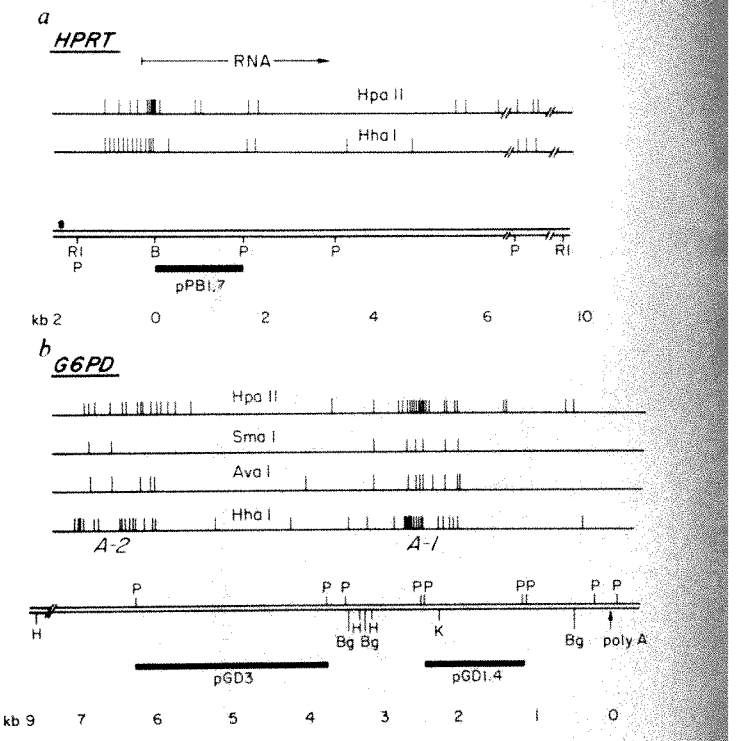


Fig. 1 Map showing relevant regions of *hprt* (*a*) and *g6pd* (*b*) loci and the cloned sequences used as probes. B, *Bam*HI; Bg, *Bgl*I; RI, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I.

The 5′ *hprt* cluster is hypersensitive to digestion not only by *Msp*I, but also to DNase I and S_1 nuclease (Fig. 3). Moreover, the region of sensitivity not only coincides with the cluster, but corresponds to the region within the cluster which contains the greatest number of CpGs. The fragments resulting from cleavage of chromatin by DNase I and S_1 (and DNA by *Pst*I) are slightly larger than the *Bam*HI/*Pst*I fragment obtained by cleaving purified DNA (Fig. 3). Therefore, the hypersensitive site is immediately 5′ to the *Bam*HI site (see Fig. 1).

We have examined CpG clusters from *g6pd*, another X-linked housekeeping gene. It is not known if there are 5′ CpG clusters at this locus because clones from this region have not been isolated. However, there are two extensive clusters in the 3′ coding region. Figure 1*b* shows a map of the 3′ region and the two genomic probes[3] used for the analysis. In chromatin from the active gene, either in male cells (not shown) or in hybrids, both clusters are extremely sensitive to *Msp*I digestion. Sensitivity of the A-1 cluster in the active gene is shown in Fig. 4. In chromatin from female cells, not all clusters are cleaved by *Msp*I, as we observe the full-length *Bgl*I fragment in addition to the cleavage product obtained from active genes (Fig. 4). Studies of hybrid cells confirm that resistant clusters are from the inactive X; chromatin of hybrids with the inactive gene, but not the active one, is resistant to *Msp*I (Fig. 4).

The A-1 and A-2 clusters are also hypersensitive to DNase I and S_1 nuclease (Fig. 5). Moreover, as occurs with *hprt*, the

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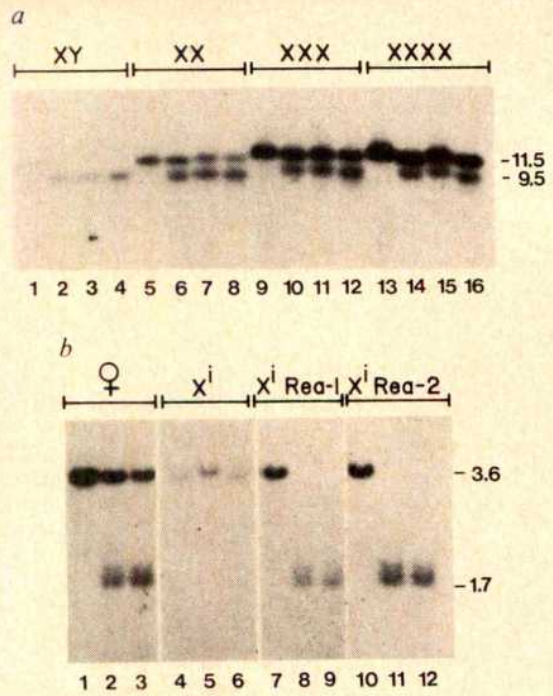


Fig. 2 *Msp*I digests of chromatin, showing sensitivity of the clusters in active *hprt* genes and resistance in inactive ones. *a*, Lymphoblasts. Lanes 1–4, 46 XY; lanes 5–8, 46 XX; lanes 9–12, 46 X/isodicentric X; lanes 13–16, 48 XXXX. Samples were treated with 0, 0.1, 0.3 or 1.0 U *Msp*I per μg DNA (from left to right in each group). Purified DNA was cut with *Eco*RI and probed with pPB1.7. *b*, Fibroblasts. Lanes 1–3, human 46 XX; lanes 4–6, hybrid G1×A9 with inactive human X; lanes 7–9, X Rea-1, spontaneous *hprt* reactivant of G1×A9; lanes 10–12, X Rea-2, 5-azacytidine-induced *hprt* reactivant of G1×A9. The hybrids and reactivants have been described elsewhere[2]. Samples were treated with 0, 0.5 or 1.5 U *Msp*I per μg DNA (from left to right in each group). DNA was cut with *Pst*I and probed with pPB1.7. Numbers on the right refer to molecular size markers in kb.

Methods. Chromatin was obtained from nuclei of lymphoblasts and fibroblasts. Lymphoblast cultures, collected at a density of 10^6 cells ml^{-1}, were washed in 0.9% saline and suspended (3×10^7 cells ml^{-1}) in RSB (10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris-HCl *p*H 7.5) supplemented with 1 mM phenylmethylsulphonyl fluoride (PMSF) and lysed by adding NP40 to a concentration of 0.2%. Nuclei from human dermal fibroblasts (or human/mouse hybrids) were purified as follows. The cells were trypsinized and resuspended in growth medium with 15% fetal calf serum, washed with 0.9% saline and treated with RSB for 10 min at 4 °C. Triton X-100 was added (to 0.2%) and the cells were lysed by Dounce homogenization to release the nuclei; then PMSF was added to a concentration of 1 mM. Nuclei were purified by washing in 50 ml RSB with 0.1 mM PMSF and resuspension in the same buffer so as to bring the concentration of nucleic acid to 250–500 μg ml^{-1}. Incubations with nuclease were carried out for 20 min at 37 °C. Reactions were stopped by quickly bringing the samples to 0 °C. The nuclei were pelleted and resuspended in 0.15 M NaCl, 0.01 M EDTA, 0.1 M Tris-HCl *p*H 7.5 and the DNA was purified by standard procedures[2].

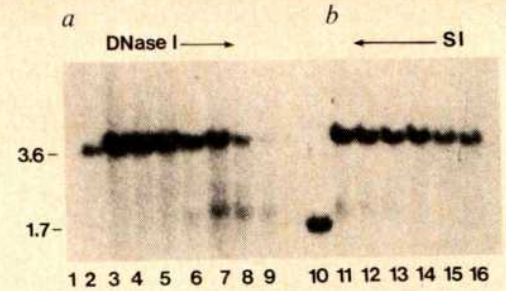


Fig. 3 DNase I and S_1 nuclease digests of chromatin showing hypersensitivity of the *hprt* 5′ CpG cluster in active genes. Lymphoblasts (46 XY) were prepared as described in Fig. 2 legend. For S_1 digests, nuclei were suspended in S_1 buffer (3 mM $ZnCl_2$, 30 mM NaCl, 0.2 mM EDTA, 30 mM sodium acetate *p*H 4.5) rather than RSB. Samples were incubated with increasing amounts of DNase I (0–0.02 U per μg DNA; lanes 1–9) or S_1 (0–20 U per μg DNA; lanes 16–11) for 20 min. DNA was cut with *Pst*I and probed with pPB1.7. A *Bam*HI/*Pst*I digest of placental DNA is included as an internal marker (lane 10).

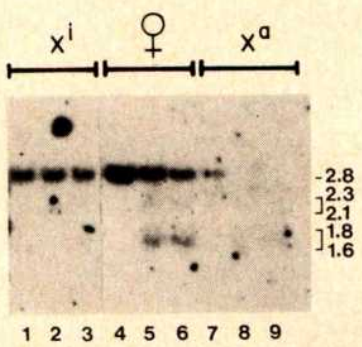


Fig. 4 *Msp*I digest of chromatin, showing sensitivity of the A-1 CpG cluster in active *g6pd* genes but resistance in inactive ones. Nuclei were prepared and digested with *Msp*I as described for Fig. 2. Lanes 1–3, human/mouse hybrid G1×A9 with inactive human X; lanes 4–6, 46 XX human fibroblast; lanes 7–9, human/mouse hybrid E×A9 with active X. Each sample was treated with 0, 0.5 or 1.5 U *Msp*I per μg DNA. DNA was cut with *Bgl*I and probed with pGD1.4.

hypersensitive regions correspond to regions having the greatest concentration of CpGs. For example, the A-2 cluster spans a 1.5-kb region, but the region of DNase I and S_1 hypersensitivity is less extensive, ~0.4 kb, deduced from the broad band (3.7–4.1 kb) representing the cleavage products (Fig. 5*c*, *d*). Furthermore, the absence of detectable DNase I or S_1 cleavage products in *Pst*I digests of the DNA probed with pGD3 (not shown) indicates that the sensitive region does not extend very far into the pGD3 sequence. That the hypersensitive region is confined to the densest part of the cluster is even more apparent in the A-1 cluster, for here there are two well-separated cleavage products (Fig. 5*a*); the hypersensitive sites map to the densest parts of the CpG cluster whereas the intervening region, which is relatively free of CpG dinucleotides, is insensitive.

Although both A-1 and A-2 clusters exhibit nuclease hypersensitivity, there are subtle differences between them. The relative intensity of the cleaved fragments indicates that the A-1 cluster is less sensitive than the A-2 cluster to either DNase I or S_1 (Fig. 5).

Our observations support the hypothesis that CpG clusters are involved in regulating the expression of *hprt* and *g6pd*. Like all regulatory regions identified[9], these clusters are hypersensitive to nuclease digestion and this sensitivity is concordant with expression of the relevant gene. Moreover, for both loci, the region of hypersensitivity maps to the clusters themselves rather than merely nearby, especially remarkable because DNase I usually prefers A+T-rich sequences[10].

The molecular determinants of nuclease hypersensitivity have been shown to be heterogeneous[9,11–13] and are likely to be complex. The ability to examine these CpG clusters in active and inactive homologues within the same cell provides insights into mechanisms responsible for hypersensitivity, at least at the loci examined. The differential nuclease sensitivity of homologous sites within the same cell indicates that neither *trans*-acting proteins[11] nor DNA sequences are sufficient determinants. Another component that seems to be required is hypomethylation of these clusters. We have never observed discordance between hypomethylation, nuclease sensitivity and expression of the relevant locus (refs 7, 8 and this paper).

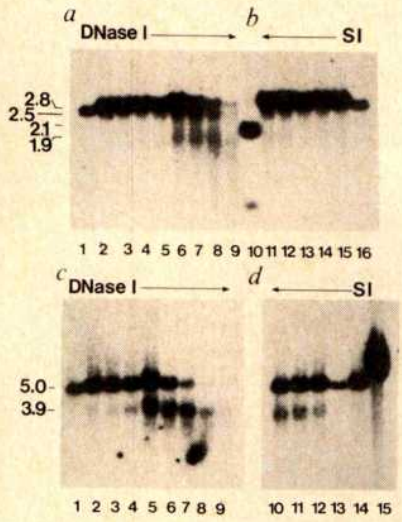


Fig. 5 DNase I (*a*, *c*) and S_1 nuclease (*b*, *d*) digests of chromatin showing sensitivity of both A-1 and A-2 CpG clusters in active *g6pd* genes. The nuclease digests of chromatin from 46 XY lymphoblasts were the same as used in Fig. 3. DNA was cut with *Bgl*I and probed with pGD1.4 (*a*, DNase I; *b*, S_1) or cut with *Hin*dIII and probed with pGD3 (*c*, DNase I; *d*, S_1). A *Bgl*I/*Kpn*I digest of placental DNA (lane 10 in *a*, *b*) is the internal marker. Lanes in *a* correspond exactly to those in Fig. 3; lanes 10–15 in *d* correspond to lanes 11–16 in Fig. 3.

The function of CpG clusters in these housekeeping genes is unknown. Although hypersensitivity of the 5′-promoter region in an active gene is not surprising, this region in *hprt* is atypical (ref. 14 and D. J. Jolly, personal communcation), lacking the characteristic regulatory signals (TATA and CAT). Thus, the 5′ cluster may provide a novel regulatory mechanism. The explanation for hypersensitivity in the 3′ region of *g6pd* is more enigmatic; however, the resemblance to the *hprt* 5′ cluster suggests that 3′ clusters may also be regulatory elements. The nuclease hypersensitivity of these CpG clusters provides the means to identify similar sequences elsewhere in the genome and the location of related clusters may provide insights into function. In any event, it is clear that CpG clusters are not unique features of X chromosomes, as they have been observed in autosomal genes[4,6]. On the other hand, these clustered CpG dinucleotides have been found most frequently in genes that are constitutively expressed, so that they may be special attributes of housekeeping genes. Such genes are unique because they are functional in all cells of the organism. The CpG-rich clusters might provide a cooperative means of minimizing the effects of chance *de novo* methylation in the regulatory regions, and/or by altering DNA structure. In conjunction with hypomethylation, these clusters could serve as irreversible switches to maintain the continuous activity of housekeeping genes.

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1. Jolly, D. J., Esty, A. C., Bernard, H. U. & Friedmann, T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5038–5041 (1982).
2. Wolf, S. F., Jolly, D. J., Lunnen, K. D., Friedmann, T. & Migeon, B. B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2806–2810 (1984).
3. Toniolo, D. *et al. EMBO J.* **3**, 1987–1995 (1984).
4. Stein, R., Sciaky-Gallili, N., Razin, A. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4035–4039 (1983).
5. Chen, M.-J. *et al. J. biol. Chem.* **259**, 3933–3943 (1984).
6. Siebenlist, U., Hennighausen, L., Battey, J. & Leder, P. *Cell* **37**, 381–391 (1984).
7. Lyon, M. F. *Biol. Rev.* **47**, 1–35 (1974).
8. Wolf, S. F. *et al. Nucleic Acids Res.* **12** (24), 9333–9348 (1984).
9. Weisbrod, S. *Nature* **297**, 289–295 (1982).
10. Dingwall, C., Lomonossoff, G. P. & Laskey, R. A. *Nucleic Acids Res.* **9**, 2659–2673 (1981).
11. Emerson, B. S. & Felsenfeld, G. *Proc. natn. Acad. Sci. U.S.A.* **81**, 95–99 (1984).
12. Jongstra, J. *et al. Nature* **307**, 708–714 (1984).
13. Schubach, W. & Groudine, M. *Nature* **307**, 702–708 (1984).
14. Melton, D. W., Konecki, D. S., Brennand, J. & Caskey, C. T. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2147–2151 (1984).

Diacylglycerol in large α-actinin/actin complexes and in the cytoskeleton of activated platelets

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The interaction of the cytoskeleton with plasma membranes may be mediated by vinculin[1–4], α-actinin[5,6] and other proteins[7–11]; α-actinin can interact specifically with model membranes only if they contain diacylglycerol and palmitic acid[12]. On stimulation of platelets by thrombin, which leads to a reorganization of the cytoskeleton[13–15], diacylglycerol is produced rapidly[16], simultaneously with the disappearance of phosphatidylinositol[16–19]. One important function of the diacylglycerol produced in platelets may be the activation of the Ca^{2+}- and phospholipid-dependent protein kinase C[20–22]. We show here that, in the presence of diacylglycerol and palmitic acid, a supramolecular complex between α-actinin and actin is formed *in vitro*. In the electron microscope, this complex displays substructures similar to those of microfilament bundles *in vivo*. Furthermore, such α-actinin/lipid complexes can also be formed *in situ* during the stimulation of blood platelet aggregation. Thus, α-actinin may be one of the proteins directly involved in structures connecting the cytoskeleton to cell membranes.

The protein α-actinin is associated with the cytoskeleton in many cells[5,23–25], including platelets[26–29]. This association is particularly prominent on physiological activation of platelets

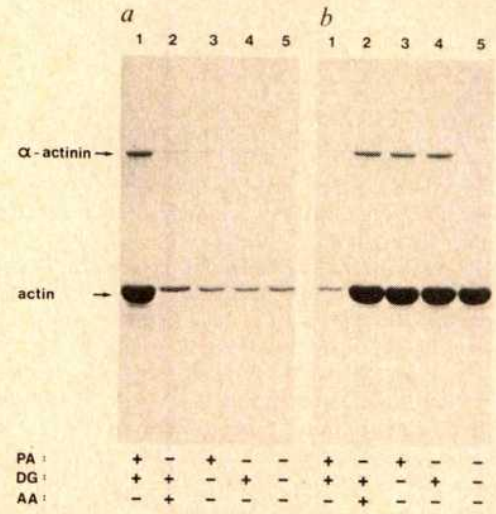


Fig. 1 SDS-polyacrylamide gel electrophoresis of α-actinin/ lipid complexes bound to filamentous actin. *a*, 10,000*g* pellets; *b*, 120,000*g* pellets. *a*, *b*, Lane 1, α-actinin and actin incubated with palmitic acid (PA) and diacylglycerol (DG); lane 2, with arachidonic acid (AA) and DG; lane 3, with fatty acid only; lane 4, with DG only; lane 5, actin incubated without lipids. *a*, Lane 1, >90% of the protein was in the 10,000*g* pellet; *b*, lane 1, <10% was in the 120,000*g* pellet. In all control experiments where the two specific lipids were absent the opposite result was observed, that is, <10% of the protein was in the 10,000*g* pellet and >90% in the 120,000*g* pellet.

Methods. 7 μg α-actinin (isolated from chicken gizzard) was preincubated with appropriate lipids for 45 min at room temperature. 55 μg G-actin (or F-actin) was then added and polymerization initiated by adding $MgCl_2$ (10 mM final concentration) and KCl (150 mM final concentration). After 30 min at room temperature, the samples were centrifuged for 5 min at 10,000 *g*. The 10,000*g* supernatants were removed and recentrifuged in a Beckman airfuge at 120,000*g*; <2% protein was left in this supernatant. Both pellets were washed and analysed on an 0.1% SDS/7.5% polyacrylamide gel[34].

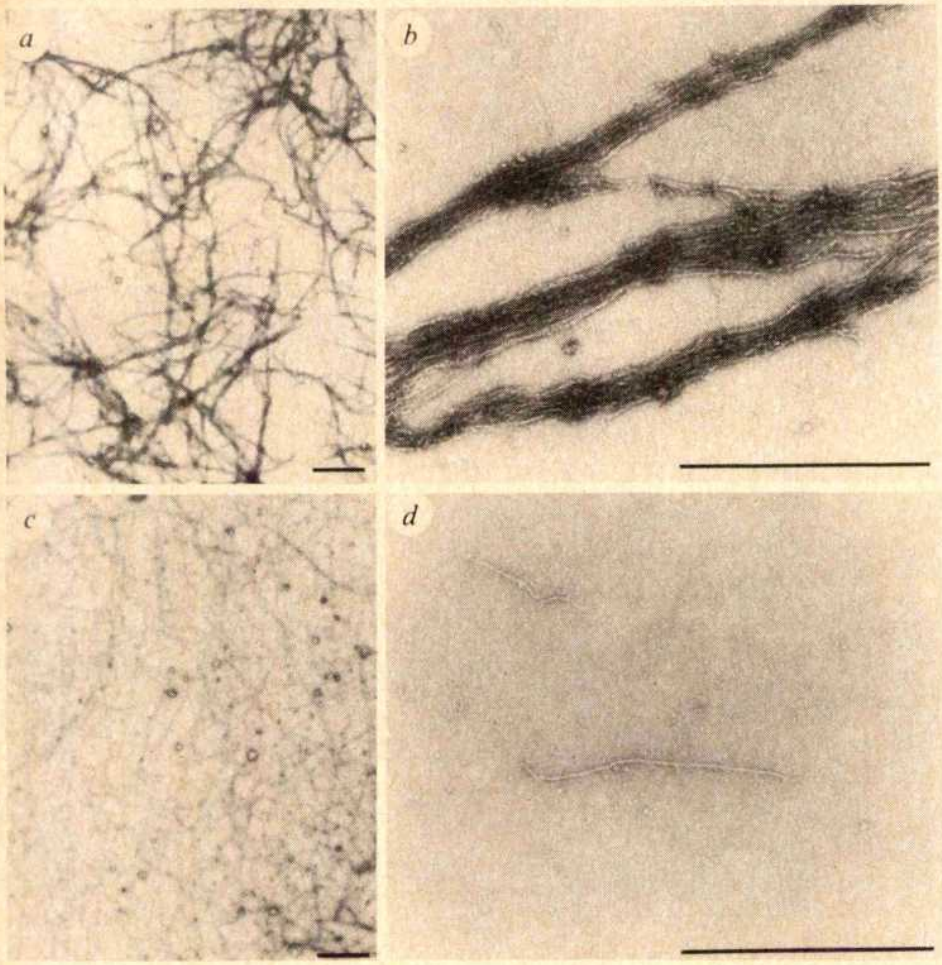


Fig. 2 Electron micrographs of negatively stained preparations of actin filaments *a*, *b*, with α-actinin, diacylglycerol and palmitic acid or *c*, *d*, with α-actinin alone. Scale bars, 0.5 μm.

Methods. Reaction mixtures containing actin, α-actinin, diacylglycerol and palmitic acid (*a*, *b*) or actin and α-actinin without lipids (*c*, *d*) were prepared and polymerized as described in Fig. 1 legend. Carbon-coated collodion grids were glow discharged and placed on a drop of particle suspension for adsorption. Samples *a* and *c* were diluted to 200 μg protein per ml immediately before being spread onto grids; samples *b* and *d* were centrifuged for 5 min at 10,000*g* as described in Fig. 1 legend. The 10,000*g* pellets were then washed twice with buffer before being spread onto grids. Adsorption of proteins was followed by post-treatment with the same buffer solution, rinsing with distilled water and staining with 2% uranyl acetate (*p*H 4.2). Specimens were examined in a Zeiss 109 electron microscope.

with thrombin[26], but the association of α-actinin with the cell membrane is less clearly understood. Some studies suggest that α-actinin is not in direct contact with the membrane[3,30,31], whereas other indicate that it is in close association with the cellular membranes of various nucleated cells[5,6] and the plasma membrane of platelets[26,32].

Recently, it has been shown that α-actinin forms a highly specific stoichiometric complex with two lipid components of the entire lipid extract of yeast cells[12]. These complexes always contain one molecule of diacylglycerol, one fatty acid of a certain type (for example, palmitic acid) and one α-actinin molecule. The need for these three components is absolute and has been demonstrated in monolayers lacking either of the two lipids[12].

Our results suggest that fatty acids, together with the diacylglycerol formed during thrombin activation of platelets, interact with α-actinin and may affect its location either in or at the plasma membrane, as well as its association with cytoskeletal actin filaments. Diacylglycerol may serve as a signal for the transmembrane control of protein phosphorylation via the activation of a novel protein kinase[21,22]. Similarly, diacylglycerol and fatty acids formed in the membrane during platelet activation may serve as a transmembrane signal for the redistribution of α-actinin in membrane regions of the cell and thereby the cytoskeleton.

A tight complex is formed between α-actinin, diacylglycerol and palmitic acid (but not between α-actinin diacylglycerol and arachidonic acid)[12]. Here we show, first, that this complex formation leads to large aggregates of α-actinin (from smooth muscle) and actin of the type observed in actin filament bundles, and second, that this complex is actually formed *in situ* during platelet activation.

The addition of α-actinin, diacylglycerol and palmitic acid to filamentous actin results in binding of the tri-component complex as well as aggregation of these actin/tri-component complexes. These large aggregates form a pellet when spun at 10,000*g* when the appropriate lipids, such as palmitic acid and 1,2-diolein, are present (Fig. 1, lane 1). Similar incubations of actin and α-actinin with other components (see Fig. 1) result in no such aggregation. This suggests that reorganization of the filamentous actin into larger complexes *in vitro* takes place only in the presence of the α-actinin/palmitic acid/diacylglycerol complex and is not just a general lipid effect. Similar results were obtained when the complex was first added to globular (G) actin followed by the induction of polymerization.

Electron micrographs (Fig. 2) confirmed these sedimentation results. A network of actin filament bundles was observed in the presence of diacylglycerol and palmitic acid (Fig. 2*a*)) whereas in the absence of lipids the network contained only single filaments (Fig. 2*c*). The 10,000*g* pellets of these preparations collected in the presence of lipids showed large actin filament bundles (10–40 filaments), whereas pellets formed in the absence of the appropriate lipids displayed an occasional single filament (Fig. 2*b*, *d*, respectively).

These results suggest that the formation of diacylglycerol and the appropriate fatty acid in membranes and their subsequent binding to a fraction of α-actinin affect the microfilaments in the cytoskeleton. To confirm whether such a complex is formed *in situ* and to demonstrate its biolgoical significance, we studied its formation in blood platelets.

Washed human platelets were labelled with ^{3}H-palmitic or ^{3}H-arachidonic acid, activated with thrombin and the cytoskeleton of resting and activated cells was isolated. The binding of lipid components to the cytoskeleton of activated platelets labelled with palmitic acid is six times higher than that of control platelets labelled with arachidonic acid. The relative binding of lipids to the cytoskeleton of activated palmitic acid labelled platelets is even more significant, considering the higher incorporation of arachidonic acid into resting platelets (74% of the total added, compared with 40% for palmitic acid), and also the higher incorporation of arachidonic acid into phospholipids

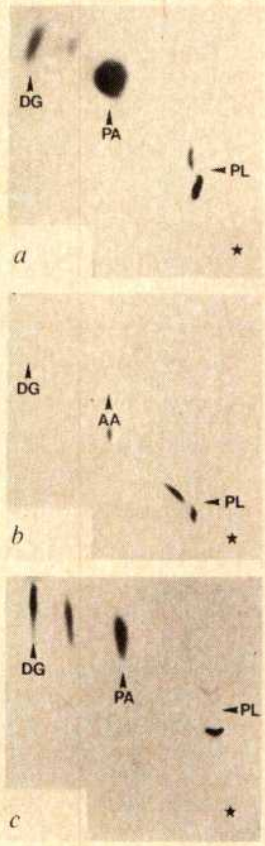


Fig. 3 Autoradiograms of lipids extracted from cytoskeletons or from immunoprecipitated α-actinin from cytoskeletons of thrombin-activated platelets analysed by two-dimensional TLC. *a*, Lipids extracted from cytoskeletons of ^{3}H-palmitic acid labelled and thrombin-activated platelets; *b*, lipids extracted from cytoskeletons of ^{3}H-arachidonic acid labelled and thrombin-activated platelets; *c*, lipids extracted from material immunoprecipitated by anti-α-actinin rabbit antiserum from dissolved cytoskeletons of ^{14}C-palmitic acid labelled and thrombin-activated platelets.

Methods. Platelets were prepared by a modified procedure of Schmidt and Rasmussen[27,33]. The washed platelets (3×10^{8} cells ml^{-1}) were incubated at 23 °C with ^{3}H-palmitic acid or ^{3}H-arachidonic acid (30 μCi ml^{-1}) in Ringer/citrate/dextrose buffer (RCD) (1.6 mM $CaCl_2$, 3.9 mM KCl, 107.8 mM NaCl, 0.5% glucose and 0.62% trisodium citrate, *p*H 7.4) for 2 h. The cells were centrifuged at 10,000*g* for 4 min and the pellet washed three times with RCD buffer. Cytoskeletons were isolated as described by Phillips *et al.*[28] and Rotman *et al.*[26]. Thrombin-activation was performed with 1 unit ml^{-1} (final concentration) at 37 °C. After 20 s, a solution containing Triton X-100 and EGTA was added to the platelet suspension until the concentrations of Triton X-100 and EGTA were 1% and 5 mM, respectively. The suspension was stirred for a further minute, kept on ice for 30 min, and then centrifuged for 4 min at 10,000*g*. The supernatants were removed and the pellets washed twice with RCD buffer containing 1% Triton X-100 and 5 mM EGTA and once with RCD buffer only. The lipids were extracted from the cytoskeletons or the immunoprecipitated α-actinin (as described in Fig. 4 legend) by the method of Bligh and Dyer[35] and separated by two-dimensional chromatography[12] on high performance TLC plates (Merck). The first dimension (upward) was in chloroform/methanol/H_2O (65:25:4). The second dimension (to the left) was in *n*-hexane/chloroform/methanol/diethylether (90:65:25:10). Radioactive profiles were obtained by exposing Kodak XS-5 X-ray film to the dried plates at −80 °C for 7 days (*a*, *b*) or 1 day (*c*). Similar results were obtained when the cytoskeletons were prepared without Triton X-100. Here, the platelets were sonicated and the pellet (1,000*g*) used for immunoprecipitation and lipid analysis. Arrowheads, phospholipids (PL), palmitic acid (PA), arachidonic acid (AA) and diacylglycerol (DG). Asterisk, origin of chromatography.

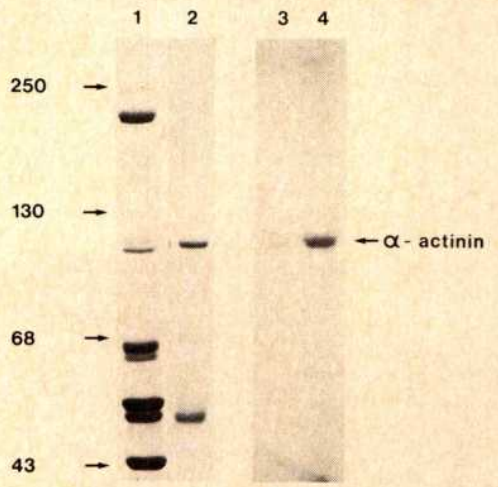


Fig. 4 Analysis of material immunoprecipitated by anti-α-actinin rabbit antiserum from cytoskeletons of thrombin-activated, ^{3}H-palmitic acid labelled platelets. (0.1% SDS/7.5% polyacrylamide gel electrophoresis). Lane 1, Coomassie brilliant blue staining of cytoskeletons (Triton X-100 residue) from thrombin-activated platelets. Lane 2, Coomassie brilliant blue staining of material immunoprecipitated by anti-α-actinin rabbit antiserum from cytoskeletons of thrombin-activated platelets. Lane 3, fluorogram of cytoskeletons (Triton X-100 residue) from ^{3}H-palmitic acid labelled and thrombin-activated platelets. Lane 4, fluorogram of material immunoprecipitated by anti-α-actinin rabbit antiserum from cytoskeletons of ^{3}H-palmitic acid labelled and thrombin-activated platelets. Numbers represent relative molecular mass, ×1,000.

Methods. Platelets and cytoskeletons were prepared as described in Fig. 3 legend. For immunoprecipitation, isolated cytoskeletons from ^{3}H-palmitic acid labelled platelets were dissolved in 1% SDS and then heated for 3 min at 95 °C. After dissociation in SDS, the sample was centrifuged for 4 min at 10,000*g*. The clear supernatant fraction was diluted with TNET-buffer (50 mM Tris-HCl, *p*H 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100) to give final concentration of 0.1% SDS. The diluted suspension was pre-cleared with 100 μl of a 10% w/v suspension of glutaraldehyde-fixed *Staphylococcus aureus* cells for 45 min at room temperature. Then, 200 μl anti-α-actinin rabbit antiserum was added and the mixture gently shaken for 16 h at 4 °C. Immune complexes were adsorbed with a 10% w/v suspension of glutaraldehyde-fixed *S. aureus*[36] for 60 min at room temperature, washed, eluted and analysed[34]. For fluorography, the gel was soaked in 200 ml of 1 M sodium salicylate[37] for 20 min immediately after electrophoresis and exposed for 4 weeks. To minimize any loss of ^{3}H-palmitate label, SDS-polyacrylamide gels were not fixed and stained after electrophoresis[38].

for the same time interval (90% of the total uptake, compared with 3% for palmitic acid).

The relative increase of radioactive lipids in the cytoskeleton of activated platelets (labelled with ^{3}H-palmitic acid) compared with that of resting platelets was at least 30-fold, indicating that physiological activation leads to a sizeable and specific attachment of lipids to the cytoskeletal elements. The low binding of radioactive lipids to the cytoskeleton of resting platelets may result from a small population of activated platelets in the preparation.

Cytoskeletons from activated platelets pre-labelled by either ^{3}H (or ^{14}C) palmitic acid or ^{3}H-archidonic acid were isolated and the radioactively labelled lipids attached to the cytoskeletons analysed by high performance TLC (Fig. 3). Most of the radioactivity attached to the cytoskeleton of palmitic acid labelled platelets came from palmitic acid, diacylglycerol and phospholipids (Fig. 3*a*). The ratio of labelled palmitic acid to diacylglycerol was not 1:1, probably because some of the palmitic acid was attached to other cytoskeletal elements than α-actinin. In arachidonic acid labelled platelets, the radioactivity associated with the cytoskeleton was mainly in phospholipids, less in free diacylglycerol and practically none in free fatty acid (Fig. 3*b*). In this case, 90% of the total radioactivity

in the platelets were incorporated into phospholipids; however, the attachment of labelled lipids to the cytoskeleton was sixfold less than that of palmitic acid labelled platelets.

Further evidence that the lipids in the cytoskeleton are bound to α-actinin was obtained by the immunoprecipitation of α-actinin from solubilized cytoskeletons of ^{14}C-palmitic acid labelled cells by anti-α-actinin antibodies. Analysis of the lipids attached to this immunoprecipitate by two-dimensional high performance TLC shows that mainly diacylglycerol and palmitic acid are attached to the α-actinin (Fig. 3c), the ratio of these lipids being almost 1:1. Furthermore, this precipitate, when analysed by SDS-polyacrylamide gel electrophoresis and fluorography, shows a clear band of radioactivity in the position of α-actinin (Fig. 4). Gel filtration and chromatofocusing give further evidence of tight binding of radioactive lipid components to protein with relative molecular mass and isoelectric point values similar to α-actinin (data not shown).

Our results show that at least 30-fold more lipids (diacylglycerol and palmitic acid) are attached to the cytoskeleton during platelet activation and that diacylglycerol is associated tightly with α-actinin immunoprecipitated from cytoskeletons of activated platelets. Neither the molecular mechanism nor the significance of this process are yet understood. Thrombin, through interaction with its membrane receptor, may initiate the cleavage of phosphatidylinositol to diacylglycerol as well as of phospholipids to fatty acids via activation of phospholipases. The mechanism by which the phospholipases are activated is not yet known. If the appropriate fatty acid is available, a stable complex will be formed between it, diacylglycerol[16-19] and the α-actinin[12] that is already associated with the membrane[26], or may now be capable of association with the membrane. At the same time, the thrombin-induced activation leads to rapid actin polymerization and reorganization of the cytoskeleton[13,27]. Such a complex of diacylglycerol, fatty acid and α-actinin can increase the affinity and attachment of membrane-associated α-actinin with the cytoskeleton[26] and is therefore fundamental in the interaction between the cytoskeleton and the membrane. In addition, the interaction of α-actinin/lipid complexes among themselves, suggested by the monolayer complexes found[12], may initiate bundling of actin filaments in membranes. Provided that the appropriate lipids are available, a similar mechanism may be involved in restructuring microfilaments in the cytoplasm. As none of the components (diacylglycerol, fatty acids, α-actinin and actin) is unique to platelets but all are present in many other cell systems, this mechanism could be general in cytoskeleton/membrane interaction in cell-shape changes and migration.

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1. Geiger, B. *Cell* **18**, 193-205 (1979).
2. Burridge, K. & Feramisco, J. R. *Cell* **19**, 587-595 (1980).
3. Geiger, B., Tokuyasu, K. T., Dutton, A. H. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4127-4131 (1980).
4. Jokusch, B. M. & Isenberg, G., *Proc. natn. Acad. Sci. U.S.A.* **78**, 3005-3009 (1981).
5. Lazarides, E. & Burridge, K. *Cell* **6**, 289-298 (1975).
6. Jockusch, B. M. *et al. Nature* **270**, 628-629 (1977).
7. Maher, P. & Singer, S. J. *Cell Motil.* **3**, 419-429 (1983).
8. Oesch, B. & Birchmeier, W. *Cell* **31**, 671-679 (1982).
9. D'Angelo Siliciano, J. & Craig, S. W. *Nature* **300**, 533-535 (1982).
10. Burridge, K. & Connell, L. *J. Cell Biol.* **97**, 359-367 (1983).
11. Repasky, E. A., Granger, B. L. & Lazarides, E. *Cell* **29**, 821-833 (1982).
12. Meyer, R. K., Schindler, H. & Burger, M. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4280-4284 (1982).
13. Jennings, L. K., Fox, J. E. B., Edwards, H. H. & Phillips, D. R. *J. biol. Chem.* **256**, 6927-6932 (1981).
14. Carlsson, L., Markey, F., Blikstad, I., Persson, T. & Lindberg, U. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6376-6380 (1979).
15. Nachmias, V. T. *J. Cell Biol.* **86**, 795-802 (1980).
16. Bell, R. L. & Majerus, P. W. *J. biol. Chem.* **255**, 1790-1792 (1980).
17. Rittenhouse-Simmons, S. *J. clin. Invest.* **63**, 580-587 (1979).
18. Lapetina, E. G. *Trends Pharmac. Sci.* **3**, 115-118 (1982).
19. Lapetina, E. G. & Cuatrecasas, P. *Biochim. biophys. Acta* **573**, 394-402 (1979).
20. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. & Nishizuka, Y. *J. biol. Chem.* **255**, 2273-2276 (1980).
21. Nishizuka, Y. *Nature* **308**, 693-698 (1984).
22. Michell, B. *Trends biochem. Sci.* **8**, 263-265 (1983).
23. Lazarides, E. *J. Cell Biol.* **68**, 202-219 (1976).
24. Webster, R. E., Osborn, M. & Weber, K. *Expl Cell Res.* **117**, 47-61 (1979).
25. Feramisco, J. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3967-3971 (1979).
26. Rotman, A., Heldman, J. & Linder, S. *Biochemistry* **21**, 1713-1720 (1982).
27. Pribluda, V. & Rotman, A. *Biochemistry* **21**, 2825-2832 (1982).
28. Phillips, D. R., Jennings, L. K. & Eduards, H. H. *J. Cell. Biol.* **86**, 77-86 (1980).
29. Langer, B. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 432-435 (1982).
30. Geiger, B., Dutton, A. H., Tokuyasu, K. T. & Singer, S. J. *J. Cell Biol.* **91**, 614-628 (1981).
31. Burridge, K. & McCullough, L. J. *J. supramolec. Struct.* **13**, 53-65 (1980).
32. Debus, E., Weber, K. & Osborn, M. *Eur. J. Cell Biol.* **24**, 45-52 (1981).
33. Schmidt, K. G. & Rasmussen, J. W. *Scand. J. Haemat.* **23**, 88-96 (1979).
34. Lämmli, U. K. *Nature* **227**, 680-685 (1970).
35. Bligh, E. G. & Dyer, W. I. *Can. J. biochem. Biophysiol.* **37**, 911-917 (1959).
36. Kessler, S. W. *J. Immun.* **115**, 1617-1624 (1975).
37. Chamberlin, J. P. *Analyt. Biochem.* **98**, 132-135 (1979).
38. Schlesinger, M. S., Magee, A. I. & Schmidt, M. F. G. *J. biol. Chem.* **255**, 10021-10024

Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin

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There is evidence that the polymerization of actin takes place at the plasma membrane[1], and that profilactin (profilin/actin complex), the unpolymerized form of actin found in extracts of many non-muscle cells, serves as the immediate precursor[2,3]. Both isolated profilin and profilactin interact with detergent when analysed by charge shift electrophoresis, indicating that they have amphipathic properties and may be able to interact directly with the plasma membrane[4]. We demonstrate here that isolated profilin, as well as the profilactin complex, interacts with anionic phospholipids. Phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) was found to be the most active phospholipid, causing a rapid and efficient dissociation of profilactin with a concomitant polymerization of the actin in appropriate conditions. These and other observations suggest the possibility of a relationship between the induction of actin filament formation and the increased activity in the phosphatidylinositol cycle seen as a result of ligand–receptor interactions in various systems.

Many eukaryotic cells have a dense weave of microfilaments (actin filaments plus associated proteins) in close association with the plasma membrane[5], apparently lying beneath the lipid bilayer all around the cell[6,7]. The dramatic changes in surface morphology and motile behaviour often seen in response to growth hormones and other cell stimulating agents seem to reflect changes in the organization and activity of this actin filament system[8], suggesting that regulated polymerization and crosslinking of actin and subsequent translocation (possibly myosin-linked) of filament assemblies formed are important steps in cell motility[6]. The detailed molecular mechanisms involved in these steps and their control, however, are unclear. Neither the site of polymerization of actin nor the nature of the transmembrane signal(s) inducing filament formation has been identified. The fact that actin polymerization occurs at the plasma membrane suggests that the precursor form of actin may be near, and possibly interacts with, the lipid bilayer. As profilin and profilactin behave like membrane proteins on charge shift electrophoresis[4], we have investigated the possibility that they interact directly with phospholipids using a viscosimetric assay that can detect effects on the stability of profilactin and on the kinetics and extent of actin polymerization.

Actin rapidly forms filaments when exposed to elevated salt concentrations (for example 50–100 mM KCl or millimolar concentrations of $MgCl_2$). However, mammalian profilin inhibits the polymerization of the homologous non-muscle actin (β- + γ-actin) quite efficiently in these conditions. Viscosimetric studies recently completed in our laboratory (H. Larsson, unpublished results) on the recombination of intact profilin and actin isolated from calf spleen[4] have shown that in 50 mM KCl, 0.1 mM $CaCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol (DTT) (25 °C), the dissoci-

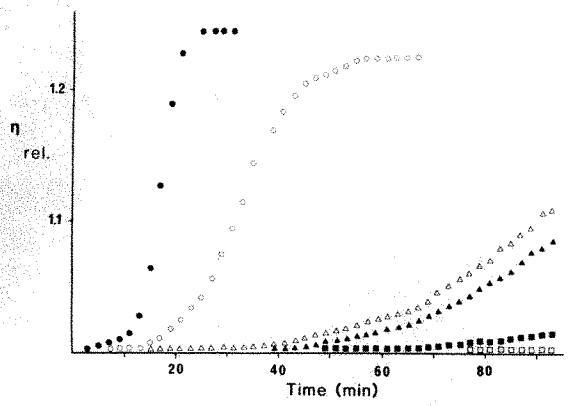


Fig. 1 Effect of various phospholipids on the stability of profilactin. Viscosity of profilactin was determined alone (□) and in the presence of PtdIns(4,5)P_2, bovine brain (●), PtdIns(4)P, bovine brain (○), PtdIns, pig liver (△), PhA, egg lecithin (▲) and PtdS, bovine (■) at various times of incubation. Phospholipid vesicles (100 μl, final concentration 0.4 mg ml^{-1}), prepared by sonication in G-buffer (5 mM potassium phosphate buffer *p*H 7.6, 0.1 mM $CaCl_2$, 0.5 mM ATP, 10 μM EDTA, 0.5 mM DTT) under nitrogen, were mixed with 429 μl of G-buffer, 8.4 μl of 1 mM EGTA, 14 μl of 4 M KCl (final concentration 80 mM) and 177 μl of profilactin-β in G-buffer (final concentration 0.4 mg ml^{-1}) in a Cannon-Manning viscometer at 35 °C (flow time 45 s). The viscosity of the incubation mixture was then measured at 2-min intervals for up to 2.5 h. In cases where polymerization had not occurred at the end of the incubation, the status of the actin was checked by adding $MgCl_2$ to 2 mM, which destabilizes profilactin. Filament formation then commences, with a lag phase of ~10 min (K_d of profilactin in 1 mM $MgCl_2$ is 4×10^{-7} M; H. Larsson, unpublished result). All phospholipids were purchased from Sigma except pig liver PtdIns, which was obtained from Serdary, London, Canada.

ation constant (K_d) of the complex is $\leq 4\times10^{-8}$ M, and that in 1:1 mixtures of the two proteins no filament formation occurs. These and previous studies on the behaviour of calf spleen profilactin[4,9,10] and on the kinetics of the polymerization of actin alone in various conditions formed the basis of our viscosimetric assay.

When profilactin-β (ref. 11) (0.4 mg ml^{-1}) was preincubated with phospholipid vesicles (0.8 mg ml^{-1}) for 15 min at low salt concentrations (5 mM potassium phosphate buffer *p*H 7.6, 0.5 mM ATP, 0.5 mM DTT) in the presence of 0.1 mM $CaCl_2$, then exposed to 80 mM KCl, all the anionic phospholipids tested destabilized the complex, causing variable amounts of the actin to polymerize. After preincubation with vesicles of phosphatidic acid (PhA) and PtdIns(4,5)P_2 the actin polymerized with a $t_{1/2}$ of 22 min. Phosphatidylserine (PtdS) was slightly less active, showing a $t_{1/2}$ of polymerization of 25 min, and PtdIns (soy bean) and phosphatidylglycerol (PtdG) were even less active ($t_{1/2}$ 35 and 37 min, respectively). The cationic phospholipids phosphatidylcholine (PtdC) and phosphatidylethanolamine (PtdE), and the diglyceride 1,2-diolein, on the other hand, were totally inactive in this respect. Very similar results were obtained with profilactin-γ prepared as described recently[11]. In controls with actin alone and with actin plus phosphatidylinositol bisphosphate (PtdInsP_2), the $t_{1/2}$ of polymerization was 21 and 17 min, respectively.

When profilactin was instead mixed with the phospholipid vesicles at an increased salt concentration, 80 mM KCl in the presence of 0.1 mM $CaCl_2$, 10 μM EDTA, 12 μM EGTA, 0.5 mM ATP, 0.5 mM DTT, 5 mM potassium phosphate buffer *p*H 7.6 (omitting the preincubation in low salt), both PtdIns(4,5)P_2 and phosphatidylinositol 4-monophosphate (PtdIns(4)P) exhibited high activities, with $t_{1/2}$ values of 16 and 35 min, respectively (Fig. 1), whereas PtdIns from pig liver and PhA gave respective $t_{1/2}$ values of 85 and 90 min; PtdS (and PtdIns from soy bean, not shown) was almost inactive ($t_{1/2}>$ 150 min). The finding that PtdIns(4,5)P_2 has no major effect on the polymerization of actin alone, and that the kinetics and extent of actin polymerization from profilactin in its presence

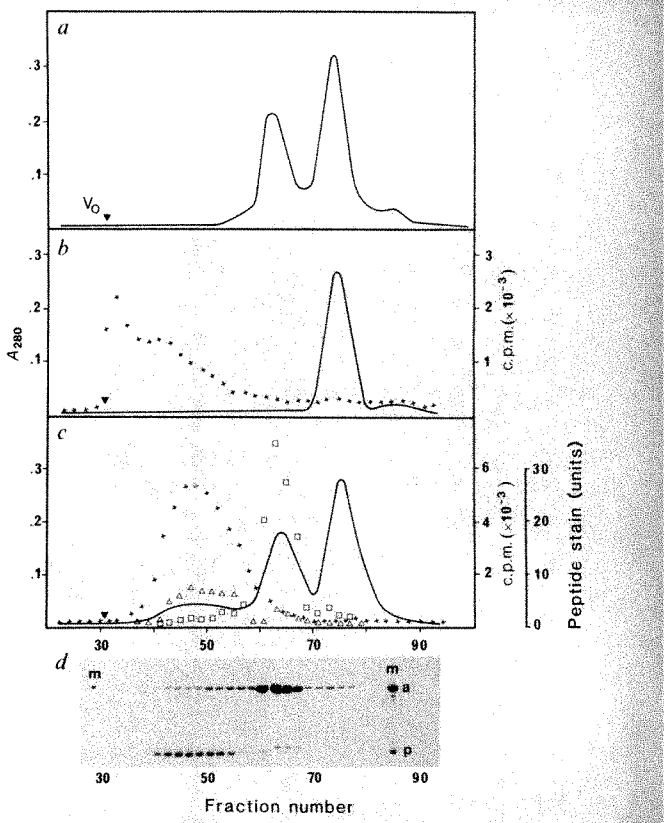


Fig. 2 The effect of PtdIns(4,5)P_2 on the chromatographic behaviour of profilactin. PtdIns(4,5)P_2 (0.8 mg; M_r 1,132)[13] was mixed with trace amounts of ^{14}C-labelled PtdIns in chloroform/methanol (4:1 and dried under vacuum. The phospholipids were then sonicated in 0.1 ml of G-buffer under nitrogen, immediately mixed with 0.4 ml of G-buffer containing 0.8 mg of profilactin-β and incubated for 10 min at room temperature, after which the incubation mixture was chromatographed at 4 °C on a column (1 × 56 cm) of Sephacryl 400 (Pharmacia) equilibrated with G-buffer (*c*). The fractions (0.5 ml per 2 min) were analysed for absorbane at 280 nm (—), radioactivity (×), profilin (△) and actin (□). To determine the relative amounts of profilin and actin, fractions of the chromatogram were analysed by electrophoresis on SDS-15% polyacrylamide gels[4] (*d*). The gels were scanned with an LKB 2202 Ultroscan after staining with Coomassie blue. Separate control runs of the same amounts of profilactin and PtdIn(4,5)P_2 are shown in *a* and *b*, respectively. In *d*, m represents marker profilactin, and a and p actin and profilin, respectively.

are closely similar to those of actin alone suggest that PtdIns(4,5)P_2 effectively eliminates the activity of profilin. (Preliminary experiments indicate that inositol trisphosphate has no effect on the stability of profilactin, but counteracts the effect of PtdIns(4,5)P_2. Furthermore, PtdIns(4,5)P_2 in mixed vesicles containing PtdIns(4,5)P_2, PtdC and PtdE in the proportions 10:45:45 is still highly active towards profilactin.)

Figure 2 illustrates the effect of PtdIns(4,5)P_2 on the chromatographic behaviour of profilactin at low salt concentrations and in the presence of 0.1 mM $CaCl_2$. In aqueous solutions, PtdIn(4,5)P_2 forms micelles of relative molecular mass (M_r) 93,000 (ref. 12). Chromatography on Sephacryl 400 alone produced low recovery of the phospholipid (20%). A large fraction of the eluted phospholipid appeared with the void volume (V_0), the rest being spread out over the first half of the chromatogram (Fig. 2*b*). When the PtdIns(4,5)P_2/profilactin mixture was analysed, however, a peak containing 50% of the phospholipid was eluted well behind the V_0. The absorbance profile obtained (Fig. 2*c*) had three major peaks, one coinciding with the phospholipid, one which was eluted at a position close to that of profilactin, and one which was eluted with the internal volume of the column. The last peak contained the ATP present in the incubation mixture. The distribution of profilin and actin over the chromatogram was determined by analysing selected fractions

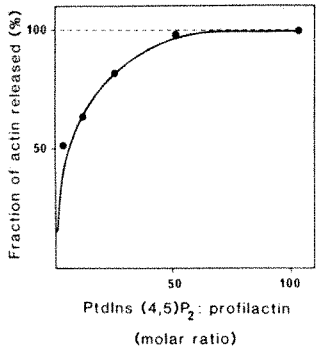


Fig. 3 Concentration dependence of the effect of PtdIns(4,5)P_2 on profilactin. Profilactin-β was mixed with PtdIns(4,5)P_2 at different molar ratios in the viscometer, and filament formation was followed as in Fig. 1. The fractions of the profilactin dissociated by the phospholipid were determined from the maximal viscosities reached, compared with that reached after addition of $MgCl_2$ (2 mM; see Fig. 1 legend).

by electrophoresis and scanning the gels (Fig. 2*d*). Gel scanning (Fig. 2*c*) indicated that the major part (88%) of the profilin co-chromatographed with the phospholipid and that 88% of the actin appeared as G-actin in the second peak. All the protein applied to the column was recovered. Thus, PtdIns(4,5)P_2 produced efficient dissociation of the profilactin complex, which apparently resulted from a relatively strong binding of profilin to the PtdIns(4,5)P_2 particles. The monomeric actin released, on the other hand, seemed unaffected by the phospholipid.

As PtdIns(4,5)P_2 clearly exerts its effect by binding to profilin, and as PtdIns(4,5)P_2 seems to have little effect on the polymerization of actin itself, it should be possible to estimate the stoichiometry of the PtdIns(4,5)P_2/profilin interaction indirectly by measuring the fraction of actin released using the viscosimetric assay at limiting amounts of PtdIns(4,5)P_2. For this, the fraction of actin released was determined by recording the maximum viscosity reached at varying concentrations of PtdIns(4,5)P_2. We found a direct relationship between the amount of PtdIns(4,5)P_2 added and the fraction of actin converted to filaments at low concentrations of phospholipid (Fig. 3), and from the initial part of the dose-response curve, calculated that dissociation of the protein complex required ~10 molecules of PtdIns(4,5)P_2 per molecule of profilactin. Considering the geometry of the PtdIns(4,5)P_2 micelles (spherical particles with a Stokes radius of 39 Å and a packing number of 82)[12] and of profilactin (Stokes radius 29 Å), this result indicates the possibility of a specific binding site for PtdIns(4,5)P_2 on profilin.

Clearly, binding of PtdIns(4,5)P_2 to profilactin causes the release of actin which is then available for polymerization in suitable conditions. Thus, the possibility should be considered that this phospholipid is involved in inducing actin filament formation in the cell. It has been realized for some time that ligand-induced turnover of phosphatidylinositol and mobilization of intracellular calcium may be causally related in many systems[13]. It was shown recently that a very early event following such receptor-mediated stimulation is the hydrolysis of PtdIns(4)P and PtdIns(4,5)P_2, resulting in the intracellular release of inositol 1,4-bisphosphate ($InsP_2$) and inositol 1,4,5-trisphosphate ($InsP_3$)[14-19]. The primary product, $InsP_3$, in turn seems to act as a second messenger for the induction of Ca^{2+} translocation[20-23]. Unstimulated cells contain a preponderance of PtdIns over the phosphorylated forms PtdIns(4)P and PtdIns(4,5)P_2, suggesting that the stimulated phosphorylation of PtdIns to PtdIns(4)P and PtdIns(4,5)P_2 through the action of membrane-associated kinase activities may be an equally early event (for review see ref. 24).

It is interesting that the increased activity in the phosphatidylinositol cycle induced by ligand-receptor interactions in turn seems to be closely correlated with an increased formation of microfilaments (polymerization of actin). Direct analysis of the G/F-actin ratio in cell extracts using the DNase I inhibition assay has shown that there is a rapid polymerization of actin following stimulation[25-30] of, for example, platelets with thrombin or ADP[25-27] and pancreatic β-cells with glucose[28-30]. In such cases, the ligand-receptor interaction has been shown to be linked to an increased turnover in the PtdIns cycle and Ca^{2+} mobilization[31-36]. Furthermore, ultrastructural studies suggest that one of the immediate events following stimulation of serum-starved glia-like cells and fibroblasts by platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) is the polymerization of actin, causing an outgrowth of new membrane lamellae and microspikes[8]; this phase is followed by increased cell motility. Both PDGF and EGF cause increased PtdIns turnover[37,38]. Finally, similar morphological changes, although less well characterized, have been observed after activation of *src* gene in cells transformed by a temperature-sensitive mutant of Rous sarcoma virus[39]; it is now known that the *src* gene product can phosphorylate PtdIns(4)P to PtdIns(4,5)P_2, suggesting its involvement in the PtdIns cycle[40].

The results presented here provide biochemical evidence for a specific effect of PtdIns(4,5)P_2 on the putative precursor of microfilaments, profilactin. This may indicate that the formation of microfilaments and thus cell motility is regulated through the PtdIns cycle.

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1. Tilney, L. G., Bonder, E. M. & DeRosier, D. J. *J. Cell Biol.* **90**, 485-494 (1981).
2. Carlsson, L., Markey, F., Blikstad, I., Pearson, T. & Lindberg, U. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6376-6383 (1979).
3. Markey, F., Persson, T. & Lindberg, U. *Cell* **23**, 145-153 (1981).
4. Malm, B., Larsson, H. & Lindberg, U. *J. Muscle Res. Cell Motil.* **4**, 569-588 (1983).
5. Poste, G. & Nicolson, G. L. (eds) *Cytoskeletal Elements and Plasma Membrane Organization* (North-Holland, Amsterdam, 1981).
6. Lindberg, U., Höglund, A.-S. & Karlsson, R. *Biochimie* **63**, 307-323 (1981).
7. Small, J. V. & L'anganger, G. *J. Cell Biol.* **91**, 695-705 (1981).
8. Mellström, K. *et al. J. Muscle Res. Cell Motil.* **4**, 589-609 (1983).
9. Markey, F., Larsson, H., Weber, K. & Lindberg, U. *Biochim. biophys. Acta* **704**, 43-51 (1982).
10. Markey, F., Persson, T. & Lindberg, U. *Biochim. biophys. Acta* **709**, 122-133 (1982).
11. Segura, M. & Lindberg, U. *J. Cell. Biol.* **259**, 3949-3954 (1984).
12. Sugiura, Y. *Biochim. biophys. Acta* **641**, 148-159 (1981).
13. Michell, R. H. *Biochim. biophys. Acta* **415**, 81-147 (1975).
14. Agranoff, B. W., Murthy, P. & Seguin, E. B. *J. biol. Chem.* **258**, 2076-2078 (1983).
15. Berridge, M. J., Dawson, M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. *Biochem. J.* **212**, 473-482 (1983).
16. Berridge, M. J. *Biochem. J.* **212**, 849-858 (1983).
17. Martin, T. F. J. *J. biol. Chem.* **258**, 14816-14822 (1984).
18. Rebecci, W. J. & Gershengorn, M. C. *Biochem. J.* **216**, 299-308 (1983).
19. Downes, C. P. & Wusterman, M. M. *Biochem. J.* **216**, 633-640 (1983).
20. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. *Nature* **306**, 67-68 (1984).
21. Burgess, G. *et al. Nature* **309**, 63-66 (1984).
22. Prentki, M. *et al. Nature* **309**, 562-564 (1984).
23. Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. Biol. Chem.* **259**, 3077-3081 (1984).
24. Berridge, M. J. *Biotechnology* **2**, 541-546 (1984).
25. Pribluda, V. & Rotman, A. *Biochemistry* **21**, 2825-2832 (1982).
26. Fox, J. E. B. & Phillips, D. R. *Nature* **292**, 650-651 (1981).
27. Casella, J. F., Flanagan, M. D. & Lin, S. *Nature* **293**, 302-305 (1981).
28. Swanson-Flatt, S. K., Carlsson, L. & Gylfe, E. *FEBS Lett.* **117**, 299-302 (1980).
29. Rao, K. M. & Varani, J. *J. Immun.* **129**, 1605-1607 (1982).
30. Laub, F., Kaplan, M. & Gitler, C. *FEBS Lett.* **124**, 35-38 (1981).
31. Rittenhouse-Simmons, S. *J. clin. Invest.* **63**, 580-587 (1979).
32. Bilah, M. M. & Lapetina, E. G. *J. biol. Chem.* **257**, 11856-11859 (1982); *Biochem. biophys. Res. Commun.* **109**, 217-222 (1982).
33. Zawalich, W., Brown, C. & Rasmussen, H. *Biochem. biophys. Res. Commun.* **117**, 448-455 (1983).
34. Cockroff, S., Bennet, J. P. & Gomperts, B. D. *FEBS Lett.* **110**, 115-118 (1980).
35. Volpi, M., Yassin, R., Naccache, P. H. & Shâafi, R. I. *Biochem. biophys. Res. Commun.* **112**, 957-964 (1983).
36. Hasegawa-Sasaki, H. & Sasaki, T. *J. Biochem., Tokyo* **91**, 463-468 (1982).
37. Habenicht, A. J. R. *et al. J. biol. Chem.* **256**, 12329-12335 (1981).
38. Sawyer, S. T. & Cohen, S. *Biochemistry* **20**, 6280-6286 (1981).
39. Boschek, C. B. *et al. Cell* **24**, 175-184 (1981).
40. Sugimoto, Y., Whitman, M., Cantley, L. C. & Eriksson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2117-2121 (1984).

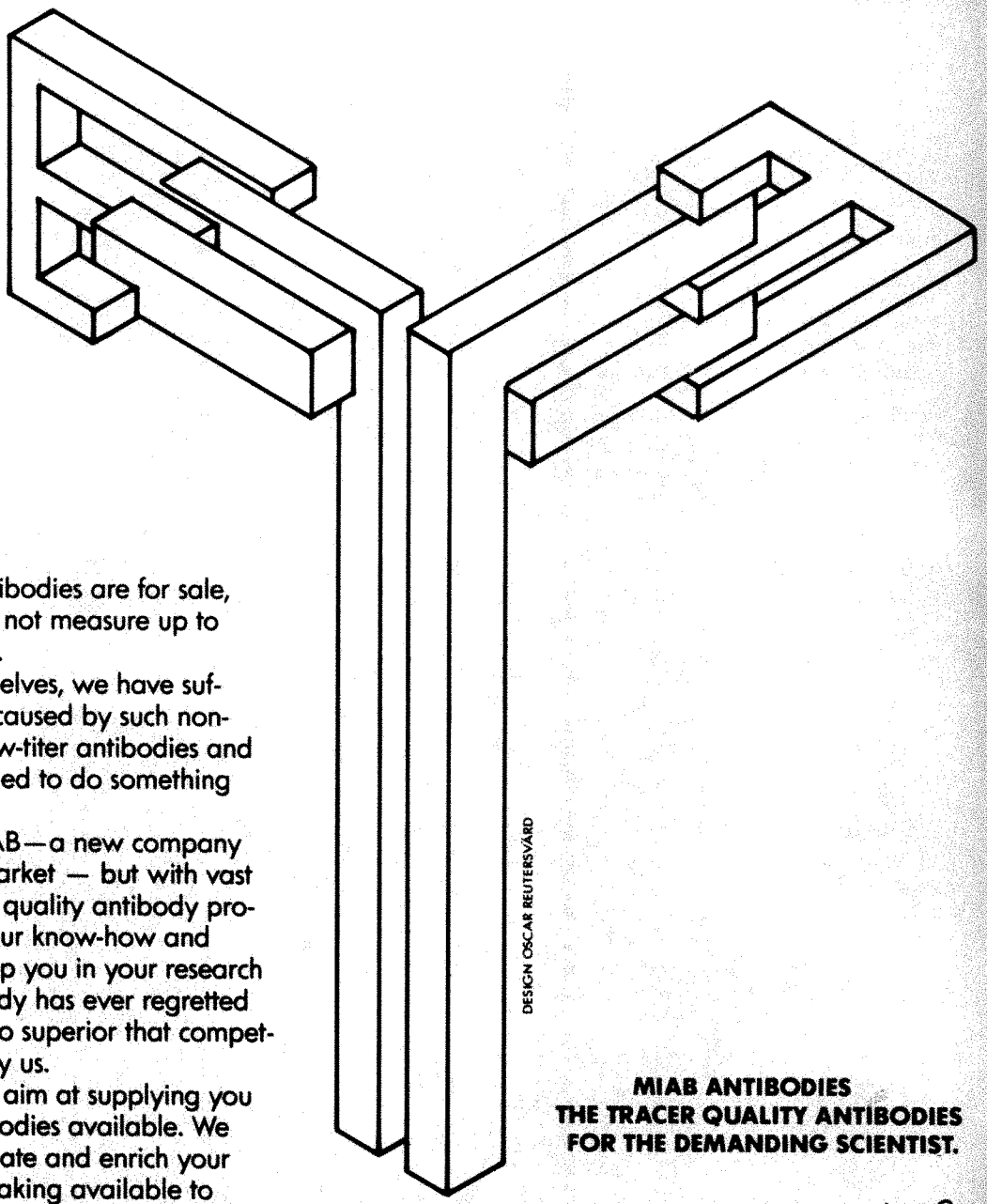
Erratum

Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor

T. Shirai, H. Yamaguchi, H. Ito, C. W. Todd & R. B. Wallace
Nature **313**, 803-806 (1985)

ON page 805, Figures 2 and 3 were transposed.

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The duplex brain

John C. Marshall

Cerebral Dominance: The Biological Foundations.
Edited by Norman Geschwind and Albert M. Galaburda.
Harvard University Press: 1984. Pp.232. $27.50, £27.50.

CEREBRAL dominance, the specialization of the left and right sides of the brain for different functions, was *almost* discovered some two millennia ago. The physicians who compiled the Hippocratic Corpus observed that injury to one side of the head was frequently associated with paralysis of the opposite side of the body; they further noted that loss of language was usually associated with *right* hemiparesis. For us, the conclusion that the left hemisphere is dominant for language processing seems inescapable. Yet neither the Greek doctors nor the many later European neurologists who made similar observations did draw this conclusion. It was not until the 1860s that Paul Broca convinced the scientific community that "On parle avec l'hemisphère gauche". From La Salpetrière in Paris, Broca reported a series of autopsied cases of aphasia with relatively focal left-hemisphere lesions and argued that the dominance of the left half of the brain was expressed in its control of both language and handedness.

Two questions therefore arose. How are the brains of left-handed people organized? And what does the right (non-dominant) hemisphere do? Broca thought that the left-handed brain was simply the mirror image of the "normal" brain. We now know that this is not the case; most left-handers have left hemisphere dominance for language. Many nineteenth-century neurologists also seem to have believed that the right hemisphere of right-handers was merely a spare, functionless entity that only came into use by "taking over" the functions of the left, should that hemisphere be damaged. This claim likewise proved false when, in 1876, the English neurologist John Hughlings Jackson showed that the right hemisphere played the leading role in "visual ideation". Thus arose the modern notion of "complementary specialization".

Over the succeeding century a vast body of work has correlated particular functional deficits with damage to more or less precisely defined regions of the brain. Yet the question of *why*, for example, the left hemisphere is (usually) dominant for language was seldom broached. In the past 15 years, however, a small group of investigators has mounted a vigorous effort to discover the biological foundations of complementary specialization in the duplex brain. The volume under review presents a somewhat manic survey of this work, a survey in which eagerness sometimes outstrips the data, loose ends dangle precariously and counter-evidence quietly disappears. Yet if even half the ideas expressed are true, our understanding of cerebral dominance will have deepened very considerably. And if some of them can be translated into clinical practice the prospects for recovery from disabling brain damage will be immeasurably improved.

First, then, some facts. It is now beyond dispute that many adult human brains show gross anatomical asymmetries that



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The brain exposed — wood cut from De Humani Corporis Fabrica *by A. Vesalius (1543).*

reflect the size of specialized cytoarchitectonic areas (discussed in the book by Geschwind and Galaburda); some of these asymmetries can be observed *in vivo* by radiological techniques, some are present in infants and fetuses, and some have even left their mark in the fossil record as far back as Peking man (LeMay). More subtle asymmetries, the differential branching of dendritic trees, for example, may also exist (Scheibel). Many lateral asymmetries in the concentration of neurotransmitters and in susceptibility to pharmacological agents are likewise coming to light (Galaburda).

Do these asymmetries have any functional significance or are they simply random variation? That the typical direction of the asymmetry often corresponds with known behavioural specialization certainly predisposes one to the former view. Thus lesions to the left planum temporale are a common cause of Wernicke's aphasia, and this area is usually larger on the left. Direct evidence would, however, be more convincing. Sometimes that evidence is positive: Ratcliff *et al.* (*Brain and Language* **11**, 87-98; 1980) found lateral asymmetries of the posterior Sylvian branches of the middle cerebral artery that predicted language dominance as assessed by successive pharmacological inactivation of each hemisphere. Sometimes it is not: Henderson *et al.* (*Neurology* **34**, 1,086-1,089; 1984) failed to find a correlation between anatomical asymmetries of the language areas (as assessed by computed X-ray tomography) and known language dominance in two groups of patients who had, respectively, sustained aphasia after left-brain damage (the typical case) and after right-brain damage (the atypical case).

In one sense, current research suffers from an *embarras de richesses*. As more and more physical asymmetries are uncovered, the task of discovering which of them have genuine psychological significance becomes progressively harder. This issue is inadvertently highlighted in chapters by Kemper and by Duffy, MacAnulty and Schachter. Kemper and Galaburda have found multiple congenital lesions in the brains of patients with developmental dyslexia; Duffy *et al.* have found widespread electrophysiological abnormalities in the same population. The latter authors are thus led to propose that "Dyslexia pure may represent dysfunction within the entire widely distributed brain system devoted to language processing". But if this is so, we are left with the mystery of why the behavioural deficits are so specific to reading and spelling.

Animal models of cerebral dominance would no doubt help distinguish causal from merely correlational phenomena: controlled experiments with animals can supplement the study of fortuitous lesions in man. Although animals do not talk (with either hemisphere), convincing evidence of both functional and physical asymmetries is now accumulating in many non-human species.

In the rat, for example, not only are there hemispheric differences in cortical thickness (Diamond), but there is also lateralization of neuroendocrine control of grooming (Gerendai); lateralization of circling behaviour consequent upon differences in dopamine concentration in the two striata (Glick and Shapiro); and lateralization of the mechanisms involved in spatial choice, taste aversion and muricide (Denneberg). Denneberg's chapter is outstanding for the clarity with which it outlines how simple models of the logic of activation and inhibition within and between the hemispheres can be used to interpret the results of lesion experiments.

One area where animal models will play an important role concerns the effects of testosterone on the developing brain. Geschwind and Behan argue that excess

testosterone slows the rate of growth of the left hemisphere and suppresses the development of the thymus gland. They thereby attempt a unified explanation of the apparent over-representation of males and left-handers who present with developmental learning disorders and certain auto-immune diseases. This left hemisphere retardation may alter the brain's pattern of connectivity as competition for synaptic space takes place in the developing cortical plate, a process for which Goldman-Rakic and Rakic have provided an elegant experimental analysis in monkey. Nonetheless, one would like to see Geschwind and Behan's epidemiological observations replicated by independent investigators. In the past, left-handers have proved a notoriously unreliable group with respect to almost any measure of neuropsychological functioning one can think of. Curiously, Geschwind and Behan fail to mention the traditional story that left-handers are, on average, poor at visuo-spatial skills, for which right-hemisphere pathology would be implicated; they also ignore Kimura's recent evidence (*Human Neurobiology* **2**, 147-154; 1983) that left-handers, as a group, are just as left-dominant for speech representation as right-handers.

The best animal analogue of human vocal learning is to be found in bird-song. Song learning in canaries is, like human speech, "a left-sided affair" (Nottebohm). Lesion of the left hypoglossus nerve, which controls the left half of the syrinx, has a far more deleterious effect upon singing than does lesion of the right. More surprisingly, seasonal variation in the pattern of song appears to be determined by the growth of *new* neurones and synapses. Although there is currently no evidence for such synapto- and neuro-genesis in the adult human brain, Nottebohm speculates that:

> In the not-so-distant future it may be possible to treat local regions of the brain to activate genes that induce dendritic retraction and growth, that induce synaptic formation and shedding, that induce birth, migration, and differentiation of new neurons.

At present, Nottebohm's "hope for a new neurology" is science-fiction although the advances of the past decade do raise the serious possibility that the diagnosis of "irreversible" brain damage will eventually give way to a less horrendous prognosis. Such success would be a fitting tribute to Professor Geschwind, who died at a tragically early age last year. The emerging synthesis that this book displays can in large part be credited to the enthusiasm, dedication and insight with which Norman Geschwind always insisted that cerebral dominance does have demonstrable biological foundations. □

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All keyed up in the laboratory

E.G. Richards

Computers in Biology: An Introduction.
By Robert Ransom and Raymond J. Matela.
Open University Press, Milton Keynes/ Taylor & Francis, Philadelphia: 1985. Pp.150. £9.95, $19.
Microcomputers in Biology: A Practical Approach.
Edited by C.R. Ireland and S.P. Long.
IRL: 1984. Pp.324. Pbk £15, $27.
Mathematics, Biology and Microcomputers.
By A.N. Barrett.
Elsevier-Biosoft: 1984. Pp.102. £24, $36.

THE day has not quite arrived when a biologist, unaccustomed to computers, can use one as he might use a centrifuge. He must go through the laborious and daunting process of coming to terms with whatever computer is available to him, its jargon, its hardware and its capabilities, its operating system and more besides. Having done that he then must either set about learning its language and write a program for the task he has in mind (and in the process more than likely reinvent the wheel) or, if he is lucky, use software written by somebody else.

Even then he is likely to encounter many pitfalls: the program may require modification before it will run on his computer (even if it is written in a language familiar to his machine); the documentation may be inadequate or even absent; it may lack robustness and die on him with an obscure error message as a result of a mis-key after he has been entering data for the last hour; he may be lulled into a false sense of security by a program which presents him with the unadorned slope of a straight line fitted to data points which are scattered at random.

Help in coming to terms with all this is provided by Ransom and Matela in *Computers in Biology*, a short but comprehensible account of some aspects of computer science. The book first explains how computers might be useful and what is involved in using them. It goes on to introduce data structures, lists and trees and the like, and then to discuss statistical analyses using computers, computer graphics, interfacing experimental apparatus with computers and finally the simulation of models for biological processes. One appendix is used to describe briefly computer hardware as we know it today, and discuss how to choose a computer (including a section on how to deal with computer salespeople), while another introduces operating systems and the facilities they might offer. A useful glossary is also provided.

In a book of such generality, no program listings are included but the text is illustrated with many examples of how computers might be applied in various branches of biology. Even if it does not cover your pet subject, it may give you seminal ideas.

Not every research worker has access to a mainframe or a mini, and may have to contemplate choosing a microcomputer. Assistance in this confusing task is at hand in *Microcomputers in Biology*, a multi-authored work edited by Ireland and Long. The first chapter goes some way to explain some of the jargon, also offering advice on choosing a microcomputer, and the book continues with chapters on the mysteries of interfaces, the problems involved in data-logging and the possibilities inherent in computer graphics. These are useful epitomes for workers with some experience in computing but may present problems to the complete beginner. The book includes summaries of some ways in which microcomputers can be used to control apparatus or analyse data obtained from several analytical techniques: spectrophotometry, chromatography and ultracentrifugation, among them. It ends with a chapter on controlling environmental factors. There is also a chapter on doing various things with nucleic acid sequences, which, it should be noted, does not attempt to come to terms with using sequence databases (such as is available from EMBL) with microcomputers.

The book should appeal to biologists who use such techniques and have some flair for instrumentation, and who are contemplating using a microcomputer. Most chapters end with listings of programs, usually in BASIC, which run on the author's microcomputer (discs containing versions of these programs are also to be available for four popular microcomputers). It is unclear how easy these will be to use, how robust they are or to what extent they protect the user from drawing false conclusions.

Barrett's *Mathematics, Biology and Microcomputers* also provides listings of simple programs (for instance to solve quadratic equations, though many of them would be better tackled on a hand calculator) but most of his book is taken up with an introduction to BASIC, solving quadratic and cubic equations (but stopping short of iterative methods for solving general equations), matrices, coordinate geometry, curve fitting, simple mensuration and so on. It is essentially an introduction to certain parts of mathematics and as such reflects, as do the biological examples, what are presumably the author's interests. It ends with a somewhat mysterious section on the manipulation of biological data by prime numbers. Unfortunately the reference to further details of this is still in press. □

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Making good

Alan R. Lehmann

DNA Repair.
By Errol C. Friedberg.
W.H. Freeman: 1985. Pp.614. $39.95, £28.95.

WORK on oncogenes over the past three years has shown conclusively that a crucial step in the conversion of a normal cell to a cancer cell involves a specific alteration in the cellular DNA, whether this be a single base change or a chromosome rearrangement. In many (if not all) cancers, the initiating event is known to be the interaction of a carcinogenic agent with cellular DNA to produce some form of alteration or damage to the DNA. All cells have evolved a complex series of enzymatic repair pathways to protect themselves from the potentially devastating effects of such damage. The importance of these pathways is shown in a number of human genetic disorders (of which xeroderma pigmentosum is the best known example), in which a molecular defect in a DNA repair pathway may lead not only to an increased frequency of carcinogenesis, but also to disorders in the neurological, immune and vascular systems.

DNA Repair provides a comprehensive, detailed and up-to-date description of current knowledge of this subject. The format is similar to *DNA Replication* by Arthur Kornberg, and like that book Friedberg's account is likely to become a classic treatise for postgraduates and research workers.

The subject is covered in a logical manner, starting with the different types of damage produced in DNA by various carcinogens, and simple enzymatic reversal mechanisms. Early events in excision repair are covered with the emphasis on detailed enzymatic mechanisms. Following chapters deal with later steps in excision repair, and with the tolerance mechanisms whereby cells are able to cope with damage which is not removed from their DNA. In each section the relatively well characterized mechanisms elucidated in bacteria are described first, followed by the inevitably more poorly understood processes in eukaryotes. The discussions of the complicated and controversial topics dealt with in the later part of the book are clear and balanced, Friedberg taking especial care to distinguish between fact and speculation.

My only criticism of the book concerns a part of Chapter 1. Here the reader is thrown headlong into some rather subtle points to do with errors in DNA replication with insufficient introduction and definition of terms, and the section would have been better placed in a later chapter. This, however, is merely a niggle. One of the many virtues of *DNA Repair* is the depth and breadth of the literature citations, each of the nine chapters being accompanied by about 200 references; I was unable to find a single important reference which had been omitted. The diagrams are clear and uncluttered, the index is comprehensive and altogether it is difficult to see how the book could have been improved. □

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Master theses

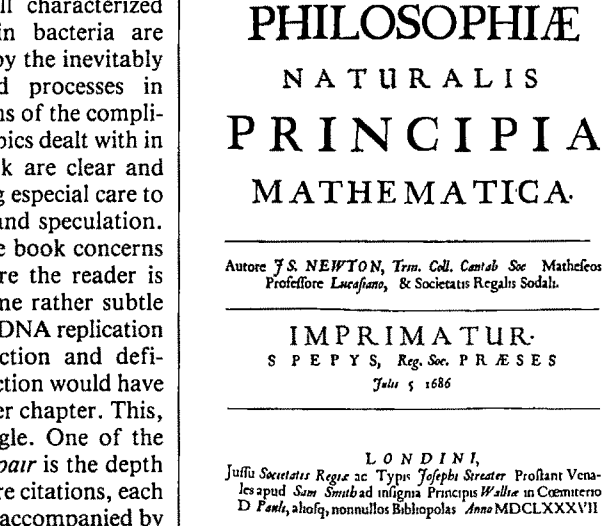
A. Rupert Hall

The Classics of Science: A Study of Twelve Enduring Scientific Works.
By Derek Gjertsen.
Lilian Barber Press, New York and Gravesend: 1984. Pp.374. Hbk $24.95, £20; pbk $15.95, £12.95.

WHAT is a scientific classic? We may all recognize the type without agreeing upon the supreme examples of it; a classic is on a pedestal. No matter that it is rarely read, and never for pleasure, it is universally admired.

Most with any claim to an opinion would include among the great scientific writers Aristotle and Archimedes (both omitted from this selection), Harvey, Newton, Darwin . . . but there comes a point when the selection of Dalton to the exclusion of Lavoisier (as in the present volume) becomes a mere matter of taste. One could certainly publish a volume parallel to Mr Gjertsen's where a different selection would possess equal scientific merit and include areas (for example cytology and particle physics) completely neglected by him. Perhaps it is natural that a British author and an American publisher should combine to display five out of twelve scientific classics written by Englishmen; indeed, after Galileo all the "classic" scientists are English with the unique exception of Linnaeus!

The conception of a "classic", therefore, if restricted to twelve or twenty or even a hundred seems devoid of precise meaning. To say that is not to judge Mr Gjertsen's treatment of twelve familiar works from the *Corpus Hippocraticum* to the *Origin of Species* as without value or interest. He has done his work well and each short study is a useful introduction to a specialist literature as well as a review of modern interpretations and critical assessments. Not everyone knows that Lyell published roughly a volume for each year of his writing life, or that the *Origin* has been translated into Latvian (besides 28 other languages). Some "classics" are better and more elaborately treated than others: the last two of them — hardly poles apart in any sense — Lyell's *Principles* and Darwin's *Origin* together rate 66 pages; the first two (Hippocrates and Euclid) 59. There are a number of small factual slips for detection by the knowledgeable, and some questionable judgements: to hold that the Hippocratic corpus is "arguably the oldest western scientific text" is very much a matter of definition, for example. But preponderantly the book conveys sound information and sensible opinions about what are, by any standards, works of outstanding importance in the history of


PHILOSOPHIÆ
NATURALIS
PRINCIPIA
MATHEMATICA.

Autore *J S. NEWTON, Trin. Coll. Cantab Soc* Matheſeos Profeſſore *Lucaſiano*, & Societatis Regalis Sodali.

IMPRIMATUR.
S PEPYS, *Reg. Soc.* PRÆSES
Julii 5 1686

LONDINI,
Juſſu *Societatis Regiæ* ac Typis *Joſephi Streater* Proſtant Venales apud *Sam Smith* ad inſignia Principis *Walliæ* in Cœmiterio D *Pauli*, aliofq; nonnullos Bibliopolas *Anno* MDCLXXXVII

Courtesy of the Royal Society

Classic beginning — the title page of Newton's Principia.

science. The format is fairly regularly maintained: author's career, context of the book, its content, its reception and its publishing history.

One might describe these twelve vignettes as basic history, and recognize that taken together they give an odd and partial view of the development of science. Presumably it was not the author's intention that a reader should form such a distorted synoptic view by reading steadily through from page 1 to page 357, but rather that he would read particular sections according to his interests and needs. Treated as a mini-encyclopaedia this is a very useful book, capable of stimulating as well as informing. But there are of course other worlds in the history of science to which this method of approach and this choice of examples give no entrance: the danger of Mr Gjertsen's book might be in concealing the existence of such worlds and the merit of exploring them. Again, he probably did not intend to use the Kuhnian language of "scientific revolutions" to recommend a "great men" account of history since his short studies often draw attention to the force of parallelism and convergence (Darwin–Wallace, for example). But in the last resort the continuity of the past — continuity so obvious to us in our own present — is its feature that most resists historical reconstruction, and cannot be promoted by a Horner-like picking out of the plums, even dried plums.

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How do enzymes work?

M.I. Page

The Bioorganic Chemistry of Enzymatic Catalysis.
By Myron L. Bender, Raymond J. Bergeron and Makoto Komiyama.
Wiley: 1984. Pp.312. £50.05, $39.50.

IT IS fashionable to justify the publication of work on some aspects of chemistry by invoking its relevance to the phenomena of life. Often this is unnecessary, because the chemistry is of interest in its own right (while sometimes the claim is exaggerated). In particular the no-man's-land between traditional disciplines is always exciting because this is where fresh understanding often emerges. The pioneering research of Myron Bender is a case in point; Bender helped to bridge the gap between chemistry and biochemistry and to define a new borderline area. Today the application of the techniques and phraseology of physical organic chemistry to enzyme catalysis is accepted practice and owes much to his work.

Our response to fundamental questions of life is usually to resort to models we think we understand. By definition, the model is a simpler system and only reflects a small part of the observed behaviour of the living system. Ill-defined separation of causes and effects *in vivo* may lead to only partial answers.

The fullness of an answer is of course determined by the question — in this case, how do enzymes work? In their book, Bender, Bergeron and Komiyama have aimed to show how an understanding of organic reaction mechanisms is relevant to an understanding of how enzymes work. They accept and propagate the view that the mechanisms — that is, the routes of bond making and breaking processes — of reactions are similar in non-enzyme and in enzyme-catalysed reactions. This is almost certainly correct. However, the number of amino acid residues of the enzyme directly involved in bond making and breaking is only a tiny percentage of the total number in the whole enzyme. What is the function of the rest of the enzyme's structure? Why can't the one or two amino acid residues involved in the "mechanism" catalyse the reaction as free amino acids? Besides enhancing the rate of reactions, enzymes are generally very discriminative in their choice of substrates. Why can't an enzyme catalyse the reaction of a compound which contains the necessary reactive function but is smaller than the normal substrate? Questions such as these are not addressed in the book.

It is not obvious that three authors have been involved in the writing, and the style throughout is uniform. This is an authoritatively written and well illustrated account of physical organic chemistry which should be useful to advanced undergraduate and graduate students wanting an understanding of the mechanism of reactions and an answer to why and how catalysis occurs. It is at its best when simple chemical reactions are discussed, but sadly fails to hint at the unsolved or controversial aspects of enzymatic catalysis. □

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Viruses in disease

Clive Sweet

Concepts in Viral Pathogenesis.
Edited by Abner Louis Notkins and Michael B.A. Oldstone.
Springer-Verlag: 1984. Pp.409. $34.40, DM 94.
Viral Pathogenesis and Immunology.
By Cedric A. Mims and David O. White.
Blackwell Scientific: 1984. Pp.398. Pbk £14.80, $26.

UNTIL recently, most virologists have shied away from studies of pathogenesis, regarding them as too difficult, less interesting or less satisfactory than the more molecular aspects of virology. Much attention has been given to the structure and biochemistry of viruses, and mechanisms of replication in cell culture, but virus pathogenicity — that is, the ways in which they produce disease — has been relatively neglected. This emphasis is changing and there is now a keen interest in the mechanisms of disease production, possibly originating from a clearer understanding of host defence mechanisms but given impetus by the merging of interests of molecular virologists and those more concerned with the causation of disease. Pioneering work with segmented RNA viruses (reoviruses and orthomyxoviruses) has led to identification of virus genes and their products and has resulted in a better understanding of virus virulence at the genetic and molecular level. The new area of recombinant DNA technology has allowed these approaches to be extended to non-segmented RNA (polio) as well as DNA (herpes simplex, pseudorabies) viruses.

While studies on virus pathogenicity hold great promise, they demand a breadth of understanding across many specialized areas. Knowledge of virus structure, genetics and replication, together with broader aspects of cell and host biology, is required for a full appreciation of the subject. Consequently, the writing of a textbook on the subject is a daunting task. The two books reviewed here have tackled this problem in different ways.

The editors of *Concepts in Viral Pathogenesis* have chosen the multiauthor approach. No fewer than 75 authors have contributed a total of 53 articles under the headings of "Host Control of Viral Spread", "Host Range and Tissue Tropism", "Virus Maturation and Diversity", "Virus Persistence", "Virus-interaction with the Immune System", "Evolving Concepts in Viral Pathogenesis" and finally "Control of Viral Disease". The book is intended to be a series of mini-reviews and editorials, 1,000–2,000 words in length, each a "pithy distillation of the state-of-the-art".

Sadly, the book does not attain these objectives. Although all of the articles contain much information they often amount only to thumbnail sketches and are too short to be really useful. However, they are generally well referenced (with one notable exception where no references are given), allowing easy access to more detailed information. Inevitably, there is some duplication between chapters, (fortunately never contradictory), and some areas are ignored. For example, there is no chapter on virus-dependent damage (cytotoxicity) and the impression gained from reading the book is that viruses do not in themselves cause disease, this being almost entirely due to aberrations in host responses. A consideration in relevant chapters of how viruses avoid, counteract or fail to induce host responses would also have been useful. Nevertheless, the book is a helpful source of reference and concepts. Typographical errors are few but the occasional use of abbreviations without definition is annoying.

In contrast Mims and White have produced an excellent text with just two contributors. Their book is packed full of information in an easily digestible, interesting and entertaining style. It covers in considerable detail the various aspects of virus pathogenesis — basic characteristics of viruses, infection and spread of viruses through the body, the immune response to viral infection, mechanisms of disease production, determinants of viral virulence and host resistance, persistent infections and finally immunization against virus diseases — from the point of view of both the host and the pathogen.

The book is especially strong in its treatment of the immune response. Indeed, the depth of coverage of all topics makes it suitable not only for medical and science undergraduates and postgraduates but also for scientists in, or moving into, viral pathogenesis research, for whom the up-to-date and extensive reference section should prove especially valuable. Clear diagrams and tables illustrate many points throughout the book and each chapter concludes with a summary and selected readings. □

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Earth Sciences

Aquatic and Terrestrial Humic Materials. R F. CHRISTMAN and E.T. GJESSING (eds) *Ann Arbor 1983. Pp. 538 ISBN 0-250-40550-4 £37.*

Future Weather: Carbon Dioxide, Climate and the Greenhouse Effect. J GRIBBIN. *Pelican 1983 Pp 272 Pbk ISBN 0-14-022459-9 £3.50*

Numerical Prediction and Dynamic Meteorology, 2nd Edn. G J HALTINER and R.T WILLIAMS *Wiley· 1983 Pp 477 ISBN 0-471-05971-4. Np*

Red Sky at Night. By JOHN BARRINGTON. *Michael Joseph. 1984 Pp 208 ISBN 0-7181-1808-1. £8.95.*

Biological Sciences

The Growth and Development of Birds. By RAYMOND J. O'CONNOR *Wiley. 1984. Pp.315. ISBN 0-471-90345-0 £20*

Handbook of Vitamins: Nutritional, Biochemical, and Clinical Aspects. LAWRENCE J. MACHLIN (ed) *Dekker· 1984. Pp 614. ISBN 0-8247-7051-X. $95 25, SwFr 211*

The Herons Handbook. By JAMES HANCOCK and JAMES KUSHLAN *Croom Helm: 1984 Pp 288. ISBN 0-7099-3716-4. £16 95*

Heterotrophic Activity in the Sea. JOHN E HOBBIE and PETER J. leB WILLIAMS (eds). *Plenum 1984. Pp 569. ISBN 0-306-41724-3 $85.*

Insect Ecology, 2nd Edn. By PETER W PRICE. *Wiley· 1984. Pp 607. ISBN 0-471-07892-1 £38 40*

Insects of the Falkland Islands. By GADEN S ROBINSON *British Museum (Natural History): 1984 Pp 38. Pbk ISBN 0-565-00955-9 Pbk £3.50.*

Insects of the World. By ANTHONY WOOTTON. *Facts on File· 1984 Pp.224. ISBN 0-87196-991-2 $17 95.*

Introduction to Sensation/Perception, 2nd Edn. By D H. McBURNEY and V. B COLLINGS *Prentice/ Hall· 1984 Pp 385. ISBN 0-13-496019-X £27 75*

The Lichen-Forming Fungi. D.L. HAWKSWORTH and D J HILL (eds). *Blackie: 1984. Pp 158 ISBN 0-216-91633-X. Hbk £16 95; pbk £7 95*

Methods of Protein Analysis. ISTVÁN KERESE (ed.). *Halsted· 1984. Pp 371. ISBN 0-470-27497-2 £39 50*

Milkweed Butterflies. By P.R ACKERY and R I. VAN-WRIGHT. *British Museum (Natural History)· 1984. Pp 425 ISBN 0-565-00893-5. Np*

The Molecular Biology of Adenoviruses 2. Current Topics in Microbiology and Immunology, Vol. 110. WALTER DOERFLER (ed) *Springer-Verlag 1984. Pp 265. ISBN 3-540-13127-2/0-387-13127-2 DM 132, $49 50*

Neurology and Neurobiology, Vol. 11A. The Neurobiology of Zinc, Part A· Physiochemistry. Anatomy and Techniques C J. FREDERICKSON, G A. HOWELL and E J KASARSKIS (eds) *Alan R Liss 1984. Pp.404 ISBN 0-8451-2712-8 £44.00*

Neurology and Neurobiology, Vol. 11B. The Neurobiology of Zinc, Part B. Deficiency, Toxicity and Pathology C J. FREDERICKSON, G.A HOWELL and E.J KASARSKIS (eds) *Alan R Liss: 1984. Pp 360 ISBN 0-8451-2713-6 £41 00*

Pathology of Skeletal Muscle. By STIRLING CARPENTER and GEORGE KARPATI *Churchill Livingstone. 1984. Pp.754 ISBN 0-443-08068-2 Np.*

Patterns of Human Heredity: An Introduction to Human Genetics. By JAMES R. BRENNAN. *Prentice-Hall· 1985 Pp 340. ISBN 0-13-654245-X. Np*

Photosynthesis. Vol. II Development, Carbon Metabolism, and Plant Productivity. GOVINDJEE (ed) *Academic· 1983. Pp 580 ISBN 0-12-294302-3 $59*

Physicochemical Biology Reviews, Vol. 4. V P. SKULACHEV (ed) *Harwood· 1984 Pp 334 ISBN 3-718-0140-0. $89 50*

Physiological Correlates of Human Behaviour, Vol. 1 Basic Issues. ANTHONY GALE and JOHN A EDWARDS (eds). *Academic· 1983 Pp 350 ISBN 0-12-273901-9. $39 50.*

The Physiology of Trematodes, 2nd edn. By J D. SMYTH and D.W HALTON. *Cambridge University Press 1983 Pp.446 Hbk ISBN 0-521-22283-4, pbk ISBN 0-521-29434-7 Hbk £30 00, pbk £11 95*

Plant Biosystematics. WILLIAM F GRANT (ed) *Academic. 1984 Pp 674 ISBN 0-12-295680-X $49 50, £35*

The Preservation and Maintenance of Living Fungi. By D SMITH and AGNES H S ONIONS *Commonwealth Mycological Institute 1983 Pp.51 Pbk ISBN 0-85198-524-6 Pbk £6 50, $12 20*

Proteinase Action. P ELODI (ed) *Akademiai Kiado, Budapest 1984 Pp 475 ISBN 963-05-3887-3 £28 25*

Recognition Proteins, Receptors, and Probes: Invertebrates. ELIAS COHEN (ed) *Alan R Liss 1984 Pp.228 ISBN 0-8451-5007-3 £29.*

Reproduction in Whales, Dolphins and Porpoises. W F PERRIN, R L. BROWNELL and D P DeMASTER (eds) *International Whaling Commission 1984 Pp 495 ISBN 0-906975-07-7. Np*

The Role of Extracellular Matrix in Development. 42nd Symposium of the Society for Development Biology R L TRELSTAD (ed) *Alan. R. Liss. 1984 Pp 662 ISBN 0-8451-1503-0 £58.00*

Safari: The East African Diaries of a Wildlife Photographer. By GUNTER ZIESLER and ANGELIKA HOFER *Facts on File. 1984. Pp.197. ISBN 0-87196-847-9 $24.95.*

Secretion: Mechanisms and Control. R.M CASE, J M LINGARD and J.A YOUNG (eds). *Manchester University Press. 1984 Pp 313 ISBN 0-7190-0975-8 £25*

Sensory Receptor Mechanisms. W HAMMAN and A IGGO (eds) *Wiley 1984. Pp.319 ISBN 9971-950-82-0 £52 90.*

Skeletal Research: An Experimental Approach, Vol. 2. ARTHUR S KUNIN and DAVID J SIMMONS (eds) *Academic· 1983 Pp 356 ISBN 0-12-429002-7 $57.*

Sketchbook of Birds. By C F TUNNICLIFFE. *Gollancz. 1984. Pp 123 Pbk ISBN 0-575-03525-0 Pbk £5 95*

Spiders of the World. By ROD and KEN PRESTON-MAFHAM *Facts on File: 1984: Pp 191 ISBN 0-87196-996-3 $17 95.*

Staphylococci and Staphylococcal Infections. Vol 2 The Organisms *In Vivo* and *In Vitro* C S.F EASMON and C ADLAM (eds). *Academic· 1984. Pp 827 ISBN 0-12-228102-0 $70*

Stratification on Tropical Forests as Seen in Leaf Structure. By I ROTH *Dr W. Junk 1984. Pp.532 ISBN 90-6193-946-1. $115, £54*

Stress, Immunity and Aging. EDWIN L COOPER (ed). *Dekker· 1984. Pp.336. ISBN 0-8247-7114-1. SwFr 166.*

Striga: Biology and Control. E S. AYENSU *et al* (eds) *International Council of Scientific Unions. 1984 Pp 216. Pbk ISBN 0-930357-01-9 Pbk $25.*

The Surface-Contact Glia. By FERENC HAJÓS and EDUARDO BASCÓ *Springer-Verlag· 1984. Pp.81. Pbk ISBN 3-540-13243-0/0-387-13243-0 Pbk DM 52, $19 40*

Themes in Biogeography. J.A TAYLOR (ed) *Croom Helm 1984. Pp.404 Hbk ISBN 0-7099-2428-3, pbk ISBN 0-7099-2428-3 Hbk £25, pbk £12 95*

***Vicia faba*: Agronomy, Physiology and Breeding.** P D HEBBLETHWAITE *et al* (eds) *Martinus Nijhoff: 1984. Pp 333. ISBN 90-247-2964-5 $53 50, £33 75*

Vitamins and Hormones, Vol. 40. DONALD B McCORMICK (ed). *Academic 1983 Pp 312 ISBN 0-12-709840-2. $39 50*

Applied Biological Sciences

Advances in Applied Microbiology, Vol. 29. ALLEN I LASKIN (ed.) *Academic 1983 Pp 282 ISBN 0-12-002629-5. $45.*

Advances in Cancer Control: Epidemiology and Research. PAUL F ENGSTROM, PAUL N ANDERSON and LEE E MORTENSON (eds) *Alan R Liss 1984 Pp 544 ISBN 0-8451-5006-5 £44.*

Advances in Neurology, Vol. 4. R.C DUVOISIN and A PLAITAKIS (eds). *Raven. 1984 Pp 286 ISBN 0-89004-958-0 $53 50*

AIDS: A Basic Guide for Clinicians. PETER EBBESEN, ROBERT J. BIGGAR and MADS MELBYE (eds) *Munksgaard 1984 Pp 313 ISBN 87-1609715-7 $40, £29*

Analgesics: Neurochemical, Behavioral, and Clinical Perspectives. MICHAEL J KUHAR and GAVRIL W PASTERNAK (eds) *Raven: 1984 Pp 351. ISBN 0-89004-793-6 $97.50*

Cell Separation. Methods and Selected Applications, Vol. 3. THOMAS G PRETLOW II and THERESA P PRETLOW (eds). *Academic· 1984 Pp.317 ISBN 0-12 564503-1 $47.50, £33.50*

Central and Peripheral Endorphins: Basic and Clinical Aspects. EUGENIO E MULLER and ANDREA R. GENAZZANI (eds) *Raven· 1984 Pp.389 ISBN 0-88167-016-2 $77*

Computers in Endocrinology. DAVID RODBARD and GIANNI FORTI (eds) *Raven 1984 Pp 361 ISBN 0-09004-368-X $70.50*

Progress in Industrial Microbiology, Vol. 19. M E BUSHELL (ed.). *Elsevier 1984 Pp 466. ISBN 0-444-42364-8. $88.50, Dfl 230*

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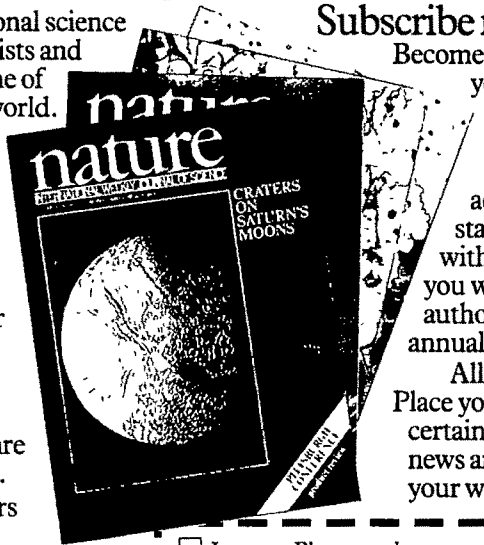
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Blanket, mask and cream

New this month are several items aimed at lab safety—including a fire-fighting blanket, a protective face mask and protective skin cream.

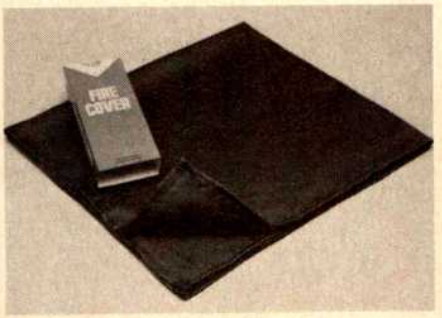
• For molecular cloning, Bio-Rad has introduced a T4 DNA ligase that can be used both to join double-stranded DNA molecules in recombinant applications and to repair single-stranded nicks in duplex DNA. Capable of ligating either cohesive-end or blunt-end DNA, the T4 DNA ligase is purified from an *Escherichia coli* strain and qualified for molecular cloning experiments by a number of quality assurance procedures. These include: monitoring ligation efficiency on both cohesive and blunt-end fragments; an endonuclease and an exonuclease activity assay and a ligation/recutting assay.
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• Celltech's range of reagents for the purification and assay of interferons now includes Beta Sepharose, a reagent based on monoclonal antibodies and intended for the purification of interferon beta. Beta Sepharose gives a single-step chromatographic process that can virtually eliminate all impurities and retain a high yield of active interferon beta.
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• A free newsletter available from Lab Safety Supply of Jamesville, Wisconsin, consists of abstracts of significant publications in the area of chemical health and safety. Subjects include personal protection, laboratory safety, safe handling, storage and disposal of toxic and hazardous chemicals, biosafety and radiation safety. For a complimentary copy of "Chemical Health & Safety Abstracts" use the Reader Service Card.
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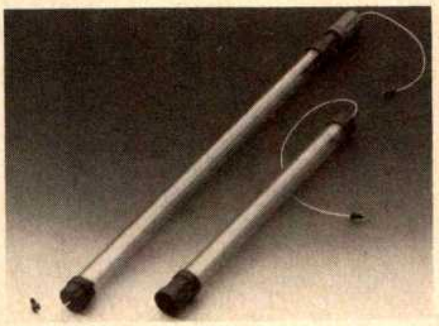
• Three leading producers of dyes and stains for microscopy have collaborated to produce a range which microscopists can rely upon to give accurate results from batch to batch. Brand-named Certistain, the new range results from an agreement between BDH Chemicals Ltd, EM Diagnostic Systems Inc., and E. Merck, on individual stain specifications covering both chemical criteria and practical biological testing. The Certistain range initially contains 30 stains and this will be expanded over the next few years to a total of around 200. Supplies of Certistain dyes and stains can be obtained from any of the three companies, their associates, agents or distributors throughout the world. A comprehensive handbook on the new Certistain range can be obtained through the Reader Service Card.
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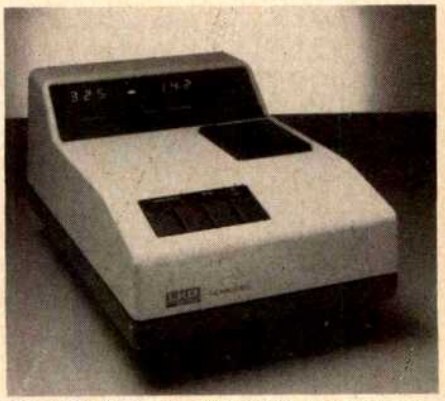
• The Fire Cover fire blanket, a completely new fire fighting aid made of fibreglass cloth impregnated with graphite and Teflon, is now available from Bel-Art Products. The blanket is capable of extinguishing small laboratory and fume hood fires, as well as Class A, light oil and grease fires. The 39″ × 43″ Fire Cover fire blanket is packaged in a small instant-access container, which can be hung on walls or stored in any convenient place. The graphite impregnated in the cover rapidly absorbs heat generated by burning material and helps lower the flashpoint below a substance's re-ignition temperature. The close weave of the cover prevents atmospheric oxygen from getting into and feeding the burning materials.
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• Bio-Yeda now offers conjugated affinity purified goat antibodies directed against mouse Fab and mouse Fc fragments. These antibodies are particularly suited as secondary reagents in work with mouse monoclonal antibodies. They may be used in hybridoma screening with the ELISA technique and as instruments for detection of monoclonal antibodies on tissue or cell preparations using indirect immunoenzymatic or immunofluorescence methods. These affinity purified anti-mouse antibodies are exhaustively adsorbed with human serum proteins to assure minimal cross-reaction with human proteins on tissue or cell preparations. The anti-mouse Fab products offer sensitive and broad activity for all mouse immunoglobulins (IgG, IgA, IgM, IgE) where the anti-mouse Fc products react specifically with mouse IgG (all subclasses). Affinity purified antibodies to mouse Fab and mouse Fc are available unmodified or as fluorescein, peroxidase and alkaline phosphatase conjugates.
Reader Service No. 106.



The LKB Novaspec for visible spectroscopy.

• For applications where a highly sophisticated spectrophotometer is unnecessary, LKB has introduced the Novaspec, a simple inexpensive visible spectrophotometer made to modern optical standards. Simplicity of operation is ensured with only four keys controlling the instrument's operation. The Novaspec features automatic wavelength calibration at switch-on, and microprocessor controlled selection of correct filter combinations. The wavelength range is broad, and a universal cell holder adds to the instrument's versatility. For applications in the teaching laboratory, the LKB Novaspec is fully complemented by a range of educational accessories, including a textbook on the basic principles of spectrophotometry, and a wall chart summarizing these principles in a graphic way.
Reader Service No. 107.

• Tecator has developed a fully automated flow injection analysis system to bring the techniques to a larger number of applications than previously possible. FIAstar is intended primarily for the fast, simple analysis of water, soil, fertilizers and beverages. This use of the FIA technique also opens up the possibility of new applications in wet chemistry analyses.
Reader Service No. 108.

• Recent additions to the Sera-Lab range include four new monoclonal antibodies against mouse T-cells for use in *in vitro* and *in vivo* depletion of T-cells and T-cell subsets. Applications include the investigation of the role of different T-cells in models for infectious disease, autoimmunity, tolerance, neoplasia and transplantation, and also in cell sorting.
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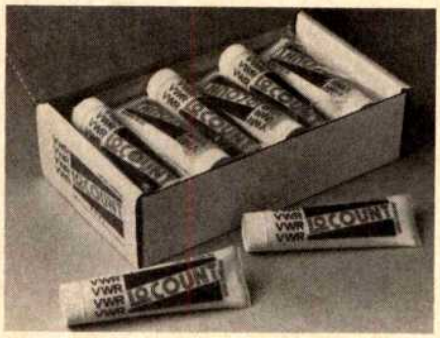
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The Gonotec automatic osmometer.

• A fully automatic osmometer using the depression of freezing point method for measurements of total osmolality in under one minute, is now available from Clandon Scientific Limited. The Gonotec Model 030 osmometer requires only 50 μl of sample (or 30 μl with lower precision) and displays results in osmol per kg. The instrument is very simple to use, being fully automatic after sample presentation. Measuring 210 × 360 × 240 mm, the 030 weighs approximately 9kg and can be connected to a recorder or printer.
Reader Service No. 110.



Locount cream protects the cleanroom environment from skin particles, and the skin from irritation.

• A new skin cream has been developed especially to solve problems with skin particulation and skin irritation in research and cleanroom environments. LoCount, from VWR Scientific absorbs right into the skin and has no particles in suspension to dry skin as oil-based creams do. The cream reduces skin particulation by as much as 90%. Its completely organic formulation means that standard cleaners easily take LoCount off any surface. LoCount is low in ionics and halogens, so if someone accidentally contaminates a device with the cream, LoCount presents minimal risk to the performance of an electrical circuit. Use of the cream reduces skin discomfort from wearing vinyl gloves for long periods, and the cream is hypoallergenic.
Reader Service No. 111.

Availability of frozen serum panels

A VARIETY of serum components have been reported to be useful in cancer diagnosis and/or in monitoring cancer treatment or recurrence. The US National Cancer Institute is interested in evaluating serum assays potentially useful in cancer diagnosis. Coded panels composed of 1 ml aliquots of pretreatment frozen sera from patients with various neoplasms, from benign disease patients, and from healthy controls are available to investigators to evaluate assays in which preliminary results indicate the ability to discriminate between cancer patients and controls. Promising results may form the basis for a subsequent grant application. Preliminary data documenting a useful test must be submitted and should include: a brief description of the assay, results in patients with cancer and in non-malignant disease, results in healthy controls and reprints of published work if available. Requests should be sent to: Diagnosis Serum Panels, Project Officer NCI-Serum Bank, Room 10A10, 5333 Westbard Avenue, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205.
Reader Service No. 112.

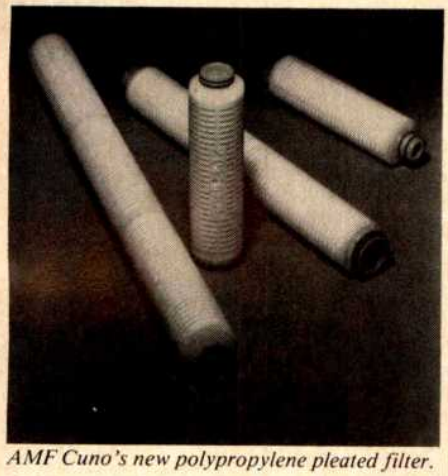
• A new range of calibrated lattices has been developed by Rhône-Poulenc. These Estapor lattices make it easier to detect the complex formed by the immunological reaction between antigen and specific antibody. Calibrated lattices, because of their high uniformity, are increasingly used in diagnostic tests such as agglutination, ELISA, RIA and erythrocyte replacement. Other applications include electron microscopy, filtration and chromatography. Estapor lattices are supplied with or without functional group and are available in either coloured, fluorescent or magnetic forms.
Reader Service No. 113.

• The Laboratory Facelet, produced by Charcoal Cloth Ltd, offers a high level of protection against toxic fumes, vapours, gases, odours and particles. The Laboratory Facelet is a derivative of the military facelet approved to NATO standards and currently in supply to the British armed forces for initial protection against chemical attack. Like the military version, the Laboratory Facelet incorporates activated Charcoal Cloth — a material composed entirely of 100% charcoal fibres which has a far higher rate of adsorption for gases and vapours than granular charcoal or charcoal-impregnated materials. In addition, other filtration materials have been included to provide a high level of protection against a particulate threat. The facelet features a low breathing resistance, a high level of speech transmission, and is light and comfortable. The mask is reuseable and much cheaper than conventional respirators.
Reader Service No. 114.



A new digital thermometer from Automatic Systems.

• Automatic Systems Laboratories, of Milton Keynes, has introduced a new digital thermometer which is capable of absolute and differential temperature measurements with a resolution of 0.001 °C between minus 270 °C and plus 630 °C. The digital readout, even at high resolution, is completely stable. The new microprocessor-controlled instrument gets its extreme stability from the use of low frequency a.c. techniques, which eliminate the thermally induced voltages and drifts associated with d.c. systems.
Reader Service No. 115.



AMF Cuno's new polypropylene pleated filter.

• The AMF Cuno Polypro is a high-surface-area, all-polypropylene pleated filter designed for critical filtration applications. Polypro cartridges are produced by pleating multiple layers of a non-fibre releasing polypropylene matrix. The 100% polypropylene construction assures compatibility with a wide range of solvents. Five absolute micrometre ratings are available from 0.6 to 10.0 μm. Applications include pharmaceutical solvents, high-purity water and photographic emulsions.
Reader Service No. 116.

• Available from Medical Systems Corporation of Green Neck, New York, The Model MF-83 Microforge is specifically designed by the Narishige Instrument Laboratory of Japan for polishing glass micropipettes used for patch clamping. The MF-83 has a magnification of 600×, and the 40× objective features a long working distance. Incorporated in the Microforge are: an adjustable lamp and heat control; an external foot switch, which controls the heating of the glass micropipettes; full manipulation of both the glass pipette and the heating element.
Reader Service No. 117.

Government Chemist opens the doors

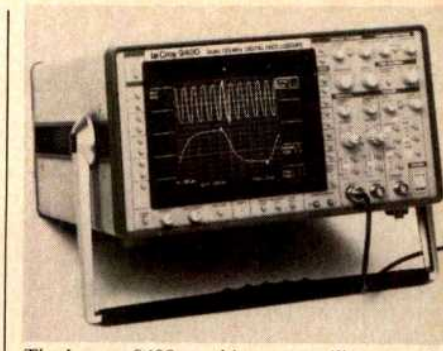
THE Laboratory of the Government Chemist, under the auspices of the UK Department of Trade and Industry, is now offering its range of materials testing facilities for contract use by the private sector. The LGC has been actively involved with materials research and development for over 20 years. Work began in the field of dentistry but has now moved into other industrial areas such as coatings technology and controlled-release. Among the facilities available at LGC are techniques for measuring:

- **Mechanical properties — including strength, hardness, roughness, abrasion resistance and fatigue.**
- **Rheological properties.**
- **Chemical and microstructural analysis.**
- **Colour measurement and translucency.**
- **Thermal properties.**
- **Environmental weathering properties.**

These facilities can be used on a regular or *ad hoc* basis for investigating the properties of polymers, ceramics, glasses, metals, coatings, rubbers, cements and dental materials and also for solving problems associated with materials in industry.
Reader Service No. 118.

• A comprehensive range of monoclonal antibodies for tissue typing is now available from Amersham International. Seven monoclonals make up Amersham's range for identifying a cell's intermediate filament protein structure. The new products are anti-cytokeratin, anti-desmin, anti-vimentin, three anti-neurofilaments and anti-glial fibrillary acidic protein. For those wishing to classify unknown tissue, Amersham offers a composite pack of basic antibodies necessary for tissue typing, while for those studying particular types of cell all the products are available separately. In addition to this new intermediate filament range, Amersham already has an established line of monoclonal antibodies for typing other intracellular structures.
Reader Service No. 119.

• The Metachem Diagnostics Cell-ect is a cost-effective system for separating cell populations without the need for expensive instrumentation. Cell-ect kits provide the reagents and instructions necessary to perform 10 separations. The principle of the system is to prepare polystyrene dishes with a specific ligand. A cell suspension is poured onto the plate and incubated. The non-adherent cells are removed by decanting the supernatant, while the adherent cells are recovered by pipetting. The kits currently available are: total histamine (TH), for the separation of all histamine receptor bearing cells; histamine receptor 2 (H_2), for the separation of Type 2 receptor bearing (H_2R^+) cells; and human Ig, murine Ig, for the separation of Ig-bearing cells from non-Ig bearing cells.
Reader Service No. 120.



The Lecroy 9400 combines an oscilloscope with the function of a transient recorder.

• The LeCroy 9400 Dual 125-MHz digital storage oscilloscope combines the advantages of an oscilloscope and a transient recorder in an easy-to-use and portable (14-kg) instrument. The 9400 features complete programmability and extensive interfacing options for remote control and computer archiving, with a wide 125-MHz analog bandwidth. Both input channels are identical, with accurate 100 megasample per second, 8-bit analog-to-digital converters and very large 32K work acquisition memories.
Reader Service No. 121.

• To facilitate studies in the field of oncogene research, Triton Biosciences Inc. is now marketing affinity-purified antibody to Ha-*ras* p21 protein. Antisera are generated in sheep against a variety of synthetic peptides corresponding to potential antigenic sites on the native protein molecule. Antibodies are purified by peptide affinity column chromatography and are characterized by Western blot and immunoprecipitation analyses.
Reader Service No. 122.



Orion's portable meter.

• Orion Research Incorporated has introduced two new meters for *p*H, DO/BOD, and redox potential measurements in the lab or field. The meters, SA 210 and SA 230, have measuring ranges of 0–14 *p*H and + 1,999 mV. The SA 210 is a *p*H/mV meter. The SA 230 is a *p*H/temperature/mV meter with ATC probe for automatic temperature compensation. Both meters come with a rugged carrying case, all the essentials needed for measuring *p*H, and a special electrode holder that attaches to the carrying case, allowing the meter to remain in the case during measurements. Battery life is approximately 100–150 hours, continuous use, and an a.c. line adapter is available.
Reader Service No. 123.

The Cambridge phenomenon

from Richard Pearson

As science parks continue to proliferate throughout the United Kingdom, it remains difficult to define the ingredients for success

HIGH technology and small businesses are seen as two essential routes to job creation in both Europe and North America. As a result, policy makers are looking for the key to unlock such job creation potential by stimulating the development and utilization of high technology, and by aiding and encouraging the would-be entrepreneur. For some, science parks are the magic catalyst which draws the two phenomena together. By observing the success of Silicon Valley in the United States, which itself was spawned by Stanford University via its science park, and now has 200,000 jobs, every higher education institution and local authority in the United Kingdom now has plans or ideas for developing its own version of a science park. For those thinking on a grander scale, then the replication of Silicon Valley itself, for example as in Silicon Glen in Scotland, which has developed as a result of public policy initiatives and now has 40,000 jobs, or the M4 sunbelt in England where growth has been more spontaneous, is the ideal translation from high technology to job creation. In the United Kingdom the concept of the science park is taking many different forms, ranging from, at one extreme, the re-labelling of a high quality industrial estate in pleasant surroundings, to the park located adjacent to and on land owned by a higher education institution. There are now over 40 such initiatives under way.

The Cambridge Science Park, formed in 1973, and owned and developed by the University of Cambridge, has been one of the UK pioneers. In addition, the area has also spawned the 'Cambridge phenomenon', the apparently spontaneous emergence of a cluster of high technology companies along the lines of Silicon Valley. As well as being the home for Sinclair and Acorn, two of the biggest microcomputer companies in the world, the area contains over 300 other high technology companies, employing nearly 14,000 people between them and accounting for one in six local jobs. Their total turnover in 1984 has been estimated at £890 million[1]. While the first high technology company to come out of Cambridge University can be traced back to 1881, when Cambridge Scientific Instrument Company was formed by Charles Darwin's son to manufacture scientific equipment for the university, the Cambridge phenonemon can be said to have really taken off some 100 years later. Over the past 20 years one new company has been established in the area every month, with peak rates of establishment occurring in 1978, 1981 and 1983 when over 2 per month were being established (Fig.1).

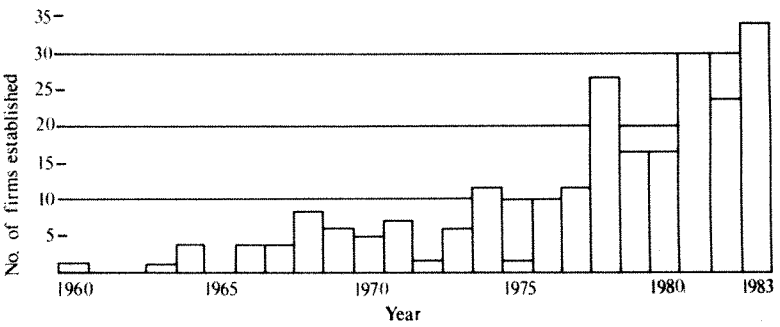


Fig.1 Dates of establishment of companies in the Cambridge area, as surveyed in ref.1.

Three quarters of these companies are new independent enterprises with the balance being made up of new branches of existing UK or overseas firms, or companies moving into the area. There is a high preponderance of very small companies in the total, one in three having five or fewer employees or an annual turnover of under £400,000. Electronics and computer hardware are the dominant business activities, but there is also a strong representation in biotechnology. A significant proportion of the companies' activities centre on research, design and development and consultancy.

The origins of the vast majority of these companies can be linked to the university, but only in a few cases, one in six, have they been formed by people coming straight from the university. For the majority it is because key staff have left one local company to start their own business in the area. This interconnection between companies, together with associated mobility of professional staff between companies, is seen as one of the great strengths and ingredients for success in both the formal settings of science parks, where the flows are expected to be between the educational establishment and industry, and in the 'Silicon Valleys', where it is between the companies involved. An additional asset of an area like Cambridge is the social network.

A distinguishing feature of high technology companies is the high qualification profiles of their workforces, a factor reinforced by the separation of manufacturing facilities to distant, and often offshore locations. In the Cambridge case, one in three of the staff in the companies were either managers or professional scientists and engineers, with the most recently formed companies having over half their staff in these categories. While shortages of such people are operating as a constraint in many locations, Cambridge does not seem to be suffering too badly. In part this is a result of the large supply of professionally trained graduates being produced by the university, but also the attractive location is an attraction to would-be migrants.

For many, the whole idea of a science park is that of the interplay of ideas between higher education institutions and local companies. In the Cambridge case, a study in 1983[2] showed that there were surprisingly few formal linkages between the companies on the park, and that companies located elsewhere in the area were just as likely to have research contracts, consultancies and teaching commitments with the university. Such linkages would seem to find more expression through informal social contacts, ease of recruitment, and a 'halo' effect which stimulates action and change by example, rather than simply as a result of close physical proximity.

What, then, are the implications for other locations seeking to encourage new high technology firms? The first is that Cambridge has many special features which are unlikely to be repeated in many other areas. The second is that such developments take time, the Cambridge phenomenon had its beginnings many decades ago, with the science park having taken over 10 years to come to full fruition, and the park itself is only one small part of the process. Finally, if US experience is anything to go by, for every success, there will be a series of still-born or struggling parks. Science parks and high technology 'clusters' may have emerged quickly in the fashion stakes; the rewards for the successful will, however, come over a much longer time span, and while initiatives by public agencies can aid this process, 'natural' advantages will be just as important, and many of these are still not well understood □

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1. *The Cambridge Phenomenon* (Seagal, Quince and Partners, Cambridge 1985).
2. Moore, B. & Spires, R. *The Experience of the Cambridge Science Park* (OECD, Paris, 1983).

nature

NATURE VOL 314 11 APRIL 1985

Towards a new world trade war?

The huge US trade deficit can be reduced in two ways—by fiscal rectitude or protectionism. There is a risk that Congress will seek the second path, which could spell disaster.

THE most remarkable modern economic phenomenon is the financial mess into which the United States has stumbled. For the past six years, since before the beginning of the Reagan administration, the federal government has been running a fiscal deficit which has increased over the years to something like $220,000 million per annum. Part of the explanation is all too familiar—the unwillingness of the government to cut public spending to match income. More recently, for the past four years, the deficit has been sustained by the Reagan administration's conviction that taxes should be cut in the interests of economic revival, and its determination that they should not even now be increased. The consequence of the deficit has been that the US government has had to borrow money in the international money markets, which has been possible only by making investment in dollar securities attractive, by high interest rates and by the promise that the domestic recovery would be strong and sustained. The consequence of that state of affairs is that the dollar itself has been "strong", which is another way of saying that the dollar has been over-valued relative to most other currencies. And the consequence of that, in turn, is that US manufacturers and farmers have been at a disadvantage compared with those elsewhere in the export markets of the world, whence the large and growing trade deficit, estimated at $137,000 million in 1984.

This state of affairs has now begun to hurt large sections of the US economy. The plight of the farmers has been clear for the past eighteen months (see *Nature* 14 March, p. 119) but is only partly attributable to the difficulties of selling US agricultural products on the international markets. Steel manufacturers have for longer been under pressure from competitors elsewhere, Europe, Japan and even some developing countries. Now the competition is beginning to hurt the traditionally strong sectors of the US economy, the general run of manufactured goods, and also those in which the United States is technically among the most advanced, electronic and telecommunications equipment for example. The glitter of the economic recovery is being dimmed by the difficulties into which some pockets of the economy have been plunged. The sharp fall in the value of the dollar (by an average of 8 per cent) against other currencies since the middle of last month will help, but only after some time and to a small extent. The fall has not yet been sufficient to correct the whole imbalance of US external trade. So, inevitably and even understandably, the US Congress is under pressure from its constituents to shield them from the unpleasant consequences of the imbalance. Last month's wild talk of a general protective duty on all imports from outside has mercifully been stilled, at least for the time being. Now, the emphasis is on correcting the trade imbalance with Japan, estimated at $35,000 million in 1984.

Reprisals

The consequences of that are odd, to say the least. On 2 April, a committee of the US Senate passed a bill that would require the federal government to take reprisals against Japan if the trade imbalance does now somehow correct itself. (The bill is not yet a law and, even if it were, its influence would be merely hortatory, but it is a sign of the way the tide of sentiment is flowing in the United States.) There has also been a flurry of bizarre diplomatic activity, with secret exchanges of special envoys between Tokyo and Washington, ostensibly seeking assurances that the newly deregulated Japanese telecommunications system will be open to equipment made in the United States, but whose political objective has been to demonstrate to the Congress that the administration is not sitting on its hands. (Even if Japan gave up the manufacture of telecommunications equipment and imported everything from the United States, the effect on the US trade deficit would be that of a drop in a bucket; the value of Japanese production in this field last year was a mere $2,300 million, less than 10 per cent of the total deficit.)

Imbalance

The issue of the trade imbalance with Japan is a red herring, but no less dangerous on that account. By any reasonable test, nobody should be surprised that if the United States has a trade imbalance on the present scale, the largest share of that should accrue to Japan, far and away the industrial economy that is the most successful at producing manufactured goods, from motor cars to electronic consumer products, which are cheap, of high quality and suited to what the international markets need. President Reagan, who is fond of telling European governments that they would be economically more successful if they were more like the United States (which may be true), should spare some time to remind US manufacturers that they should emulate their Japanese competitors.

But is not the competition from Japan unfair? This is the recurring cry, not only in the United States but also in Western Europe. And it is true that some sectors of the Japanese economy are closed to competition from elsewhere. Japanese consumers must pay more for beef than if it were imported from Texas (or Australia), for example, which has been a fierce bone of contention with the United States during the past year. But is the United States saying that the time has come when all governments, itself included, must abandon agricultural protectionism? That would be in the general interest, but no more acceptable politically in Washington than in Tokyo. For the rest, it is far from clear whether the difficulties that US manufacturers report in exporting to Japan are consequences of the supposed conspiracy of the Japanese bureaucracy against imports of any kind. But there would be serious dangers in any sustained campaign by the United States to single out Japan as the target for its chagrin over the size of the trade imbalance, not least of all the risk that the industrial community of the West, established painfully since the Second World War, may be undermined.

Whether or not competition with Japan is unfair is in any case irrelevant to the present difficulty of the United States. And everybody knows what the remedy must be. So long as the federal government's deficit is running at its present level, and while policy remains to bridge the gap by borrowing from overseas rather than by printing money (which would be inflationary), the trade imbalance will persist. So the urgent task for Congress and the administration is to cut the deficit. That is one of the issues with which the present Congress has been faced. Last week, the administration and its party's

leadership were hoping to cut $52,000 million from next year's deficit. The first thing to be said about even this ambition is that it is far too modest. The second is that it will eventually be for the Congress to decide whether the administration will get the cuts it seeks. The present preoccupation with protectionism, and with the Japanese phenomenon, is a way of hiding from the real need to cut spending or to raise taxes. Painful though these questions are, they will have to be answered at some point down the road on which the United States is travelling dangerously. □

Professional research

How to stimulate research when application is of the essence?

ENGINEERS insist that, while their professional skill is based in science (no longer exclusively physical science), that is only half the story. And similarly, physicians insist that there is more to their craft than applied biology. So much is easily accepted. But in the past several years and especially recently, there has grown up a great sense of confusion about the character and the role of research in both engineering and clinical medicine. In most places, there is a belief that suitably designed research programmes would improve the practice of engineering and medicine, and in some countries there are glowing demonstrations of what these benefits may be. But there is no general understanding of how these few successes may be replicated elsewhere, or how the universities should be most fruitfully involved.

Britain just now has encountered problems on both fronts. Thus the Medical Research Council (MRC) appears to be embarking on yet another reappraisal of its Clinical Research Centre, founded in the 1960s at least partly out of admiration for and envy of the hospital-based research carried out by the US National Institutes of Health at Bethesda (see p. 487). The British experience, unfortunately, has not been nearly as encouraging as people hoped, but for reasons that should have been anticipated.

In one sense, it is bad luck that most of the interest in the past two decades has been in the research by means of which the biological basis of medicine has been so dramatically enlarged, but MRC's clinical centre has also been frustrated in another of its original objectives, that the development of new clinical techniques and therapies should be put on a sounder basis, both by example and by the training of a new generation of research-minded clinicians. In the event, the pace of development in clinical medicine has been determined by people of the kind who have traditionally made the running, usually members of the staffs of teaching hospitals. Most often, in Britain, the costs of these developments have been borne unselfconsciously by the health services. The practical question MRC will soon have to ask is whether its influence on the direction and pace of change in medicine would be greater if more of its resources were spent on research grants, and less on in-house research. The dangers in spending money on research grants, sometimes apparent in the United States, is that the techniques of assessment by peer-review that work reasonably well in basic science are a poor guide to the selection of projects and people in the field of applications. The paper-chasing that sullies much of clinical research in the United States should be a warning of the dangers.

Similar difficulties arise in engineering research. Everybody agrees about the need that basic research should prosper (and in fields such as materials science, British support for the foundation of engineering is sadly deficient). There is also a general conviction that what is called engineering research should also prosper. In the United States, one result has been (see p. 488) that the National Science Foundation has now set up the first six of what is intended as a network of engineering research centres closely linked with universities. The hope is that these will become the well-springs of technological change in the decades ahead, but that they will also train the executors of innovation. The goal is sensible enough (and the centres closely resemble the network established in Britain over the past decade by the private charity called the Wolfson Foundation, many of whose centres have well served their purpose). But the question remains of what engineering research is like.

The point is nicely illustrated by the dilemma in which the British Science and Engineering Research Council now finds itself. For well over a decade, the council (which is the chief source in Britain of support for academic research) has been under pressure to do more to turn ideas into wealth. Its response has been to spend money on a variety of schemes for inducing collaboration between industry and universities (most of them successful and cost-effective), but also to spend more money on engineering research, whatever that may be. Direct spending in universities and polytechnics in the United Kingdom under the heading of engineering is now more than 40 per cent of the total (which is one reason why spending on, say, basic physics is being skimped). Yet a committee report, on which the research council is now seeking general opinions, would have the scale of spending further increased, with the objective of turning British higher education into one of the principal sources of technological innovation. An important part of the argument is that the acknowledged differences between science and the practice of engineering require that engineering projects should be directed not at the discovery of new knowledge as such but at the conception and design (not necessarily the development) of new products and machines. The research council is believed to be in two minds about the proposal, and even for the first time to be prepared to contemplate a separate research council for engineering.

This may be the only way to go in a country such as Britain, but everybody should be aware what is entailed. Higher education is for training students, and research (whose first product is scholarship) is also an extension of that process. Most engineering education (like most medical education, especially in Britain) neglects the basic science of the field (which may be part of the reason why British industry has been slow to exploit scientific innovation). There are several conceptual fields, such as the development of techniques for telling complicated computer systems what to do, or in definition of the relationship between the chemical and physical microstructure of a semiconductor and its properties, where it is easy to see armies of talented people providing future engineers with grist for important mills. But if it is to be the role of engineering research within a university environment to take on the precompetitive development of new products, the question must naturally arise of what the role of industrial companies' research and development departments should be. And will it be possible for universities to use their existing techniques of peer-review and the like to decide who should do what, with what resources?

Exploitation

The six new centres established by NSF should yield some first-hand experience of the problems to be solved, although there are already whole institutions such as the Massachusetts Institute of Technology which are partly organised along these lines already. In Britain, the case for following the path that seems now mapped out is that there is a need, unmet by industry, for the more deliberate exploitation of new ideas, that such a development would also lend engineering some of the distinction engineers say it lacks and that there is a need for a stronger relationship between industry and higher education on more general grounds. The arguments against are that universities may turn out to be poor managers of such novel institutions (and worse, that they might persuade academic engineers to follow inappropriate norms of academic attainment). But the essential criterion is that more support for these engineering initiatives in universities should not rob basic research of funds. □

US fusion research

Hope now depends on overseas collaboration

Washington

THWARTED by a probable 17 per cent cut in the US fusion research budget in two years, US scientists are pinning their hopes for further progress on increased collaboration with Europe and Japan. Plans have been abandoned for a US device that would both achieve plasma ignition, the point at which fusion becomes self-sustaining, and simultaneously allow the testing of high-performance technology needed for a commercial fusion reactor.

The US Department of Energy's latest magnetic fusion programme, now before Congress, makes clear that the next US machine will instead concentrate primarily on the physics of plasma ignition; no timetable is specified. Details of how the programme should be implemented are being worked on at Argonne National Laboratory. The best hope now is that, through international agreements now in the final stages of negotiation, US scientists might participate in research on high performance technology on a machine outside the United States.

Waning interest in fusion research in the United States is, according to the Department of Energy, due to the oil glut. The new plan says simply that there is no longer a premium on the early development of fusion power. Instead, the goal now should be to further scientific understanding of different options for plasma confinement. The US fusion research budget fell from $470 million in 1984 to $436 million in 1985 and the administration's proposal for the 1986 budget is $390 million. There seems to be no chance that Congress will increase this amount and, indeed, a further decrease cannot be ruled out despite lobbying efforts by the research community.

The impact of the reduction so far has been felt both in the five national laboratories where fusion research is carried out and in universities. Some job losses now seem inevitable. At the Princeton Tokamak Fusion Test Reactor, plans to introduce tritium (which would be used, along with deuterium, as fuel in a fusion reactor) have been delayed by two years, until 1988, and construction of the MFTF-B tandem mirror device at Lawrence Livermore Laboratory has been indefinitely postponed. The 1970s goal of achieving a commercially feasible fusion reactor by the year 2000 is now acknowledged to have receded by at least 25 years.

The Department of Energy's programme suggests four major areas of research for special attention. These have been agreed in principle with international partners and will be discussed at the next economic summit meeting at Bonn later in the year. The objectives are the choice of confinement system, the properties of burning plasmas (not now being investigated), the development of suitable construction materials for a future commercial reactor and the development of a blanket for extracting the energy emitted from a fusion reactor in the form of energetic neutrons.

Two international agreements are being negotiated by the Department of Energy under the auspices of the International Energy Agency. These would cover US work on the ASDEX device and the Wendelstein stellarator in West Germany. A further agreement being negotiated with the European Community would formalize US collaboration in the TORE-SUPRA device under construction in France (a steady-state tokamak). Although there have been significant scientific exchanges in the past, legal difficulties—especially over patent rights—have limited extended stays, according to John Clarke, director of the Department of Energy's fusion programme; separate bilateral agreements with Japan are already in operation.

Fusion researchers are sensitive to the suggestion that they have failed to deliver the goods to their sponsoring agencies: there are encouraging results shortly to be reported from the Princeton tokamak which put the US "well on the way" to achieving the goal of scientific break-even, according to Harold Furth, director of the Princeton Plasma Physics Laboratory. A break-even demonstration is scheduled for 1988. Furth hopes that a small US ignition device could be started in 1988, for completion five years later; experience with low-duty cycle experiments would then start in the mid-1990s. Design work is well advanced on a larger technology-testing device that could be constructed in collaboration with international partners starting in 1990 or 1991. This device, known as INTOR, has been planned by the International Atomic Energy Agency since 1978. If built, experience with high-duty cycle operations could start at the turn of the century. Whether governments will provide the necessary funds remains to be seen.

Tim Beardsley

Clinical research

UK centre under scrutiny again

AFTER one rather futile attempt to help the UK Medical Research Council (MRC) to decide the future of its Clinical Research Centre (CRC), a committee under the chairmanship of Sir Michael Stoker is to try again. The outcome should be an assessment of the first 15 years of the centre's work and is likely to include recommendations as to how CRC should be changed to meet both the changed circumstances in which it finds itself and widespread scepticism of its value.

At a cost of £10.4 million in 1983–84, CRC is the most expensive MRC research establishment. Despite a 20 per cent cut in its running expenses and no money for new equipment last year, CRC consumed nearly nine per cent of the total MRC budget. The cost per unit head is not excessive. The question now is not so much whether there are too many heads—although many would like that to be asked—as whether the centre fulfills its role.

That question can be answered only if the role is clear, but Stoker's first committee seems to have drawn a blank on this point. Nevertheless, it seems agreed that CRC was set up to be a focus and centre of excellence in clinical research in the context of commonplace diseases. That is why it shares a site and facilities with Northwick Park Hospital, an otherwise ordinary district hospital of the National Health Service. This arrangement has certain advantages but, according to Sir Christopher Booth, director of CRC for the past seven years and four years from retirement, it also has disadvantages.

One is that there has been little room for medical specialization within the hospital, although the regional health authority now seems willing to change that. A second is that the CRC director has little influence over hospital appointments although, unless those appointed have an interest in research, CRC cannot thrive. Sir Christopher also believes that CRC suffers from not being a recognized centre for postgraduate training.

To some outsiders, including many MRC scientists, the problems of CRC are greater. In 15 years, it has not made its mark as a centre of excellence and has not been quick enough to take aboard new approaches and technology. Why, in particular, is it only now that the application of molecular biology to medicine is beginning to find a place at CRC?

Stoker's second committee has the task of trying again to establish the original aims of CRC but also, and much more importantly, to advise MRC whether the objectives should now be changed and, if so, how to go about it. The committee's report is expected to reach MRC by the end of the year.

Peter Newmark

US engineering research

Six special centres founded

Washington

THE National Science Foundation (NSF) has now designated the first six of its engineering research centres, planned to operate in close association with university departments. They were proposed in 1983 by NSF's engineering directorate in response to widespread concern about the quality of engineering research in US universities. The six centres will be at: the University of California at Santa Barbara; Columbia University, New York; the University of Delaware, with Rutgers University; the University of Maryland, with Harvard University; Massachusetts Institute of Technology; and Purdue University.

NSF's proposal to designate special engineering research centres was endorsed heartily last year by the National Academy of Engineering, which urged NSF to aim for no fewer than 25 centres. NSF's director, Erich Bloch, said last week that progress towards that goal would depend on the continuing support of Congress. The administration's budget proposal for fiscal year 1986 includes $25 million for engineering centres, which should allow a further five or six centres to be designated next year.

The subject area to be covered at each centre was proposed by the host institution. NSF did not attempt to identify in advance the particular specializations it wanted to support, but merely sought to avoid duplication. The purpose of the centres, according to Bloch, is to "enhance the future competitiveness of US industry in international markets" by furnishing industry with a "solid knowledge base".

Location	Funds (over five years)	Subject
University of California, Santa Barbara	$14 million	Robotic systems in microelectronics
Columbia University	$20 million	Telecommunications
University of Delaware (with Rutgers University)	$7.5 million	Composites manufacturing
University of Maryland, College Park (with Harvard University)	$16 million	Systems research
Massachusetts Institute of Technology	$20 million	Biotechnology process engineering
Purdue University	$17 million	Intelligent manufacturing systems

The centres will together receive $94.5 million from NSF over five years, but funds will also be contributed by the host institutions. NSF hopes that its contribution will be matched by state governments and industry. The emphasis is on fostering interdisciplinary research in such areas as robotics, materials science, telecommunications, biotechnology engineering and artificial intelligence; each centre will concentrate on a particular area. Extensive collaboration with industry is expected. The centres will also be used for training, both of graduates and undergraduates; new courses

At least one US senator had lobbied NSF to locate an engineering research centre in his state, apparently successfully. But Bloch insists that siting decisions had been made exclusively on the basis of peer review of the 142 proposals from 106 institutions. **Tim Beardsley**

NASA budget freeze in prospect?

Washington

THE House of Representatives last week sent a message that it may be in no mood to go along with the Reagan Administration's programme of selective budget increases for science while other non-military portions of the budget are slashed. By a surprisingly large 369–36 vote, the House refused to approve the 5 per cent increase for the National Aeronautics and Space Administration (NASA) that the administration had requested for fiscal year 1986. Instead, if the House gets its way—the Senate has yet to act—NASA's overall budget will be frozen at its present level.

The House offered no prescription for allocating the cut among NASA's many programmes, except to say that $45 million of the $375 million reduction should come out of salaries and administrative costs.

The details could be worked out in a House–Senate conference or conceivably left to the NASA administrator.

The President's budget proposal for NASA—which had been approved largely unaltered by the House Science and Technology Committee—specified major increases for the space station (up to $220 million from the current $140 million) and for space applications and commercialization (up to $580 million from $440 million). The scientific research programmes were due for much more modest rises.

By a much closer margin, the House took a token step towards demanding that the shuttle should pay for itself. NASA had wanted to hold the maximum price for a full shuttle payload to $71 million, rather than raising prices in 1988. The House ordered the maximum price to increase in 1988 to $105 million.

Stephen Budiansky

Soviet research

Aleksandrov on self-reliance

SOVIET science must try harder to be self-sufficient, Dr Anatolii P. Aleksandrov, president of the Soviet Academy of Sciences, told the academy's general assembly last month. In some fields in which the Soviets had originally been in the lead, he said, projects had been held up for so long that, in the end, foreign equipment had to be purchased under licence and Soviet work in the field had been terminated. The very scale of Soviet industry seems to be a large part of the difficulty.

Aleksandrov is well known as an advocate of Soviet self-sufficiency. On many occasions, he has somewhat grudgingly referred to international cooperation as necessary in the interests of detente and the elimination of duplicated effort, while emphasizing that the Soviet Union needs no outside help.

Nevertheless, Aleksandrov's new criticisms of Soviet industrial performance are more than his habitual harping on self-sufficiency. His special targets have in common their concern with short-run production.

Thus, said Aleksandrov, the Soviet chemical industry, in spite of its "tremendous" scale, cannot produce the relatively small quantities of reagents needed for high-accuracy analysis. Similarly, in spite of the scale of the nuclear industry, which is capable in theory of supplying the economy with all necessary isotopes and isotope-labelled compounds, many institutes still have to purchase such products from abroad.

Similarly with precision research instruments, although the Soviet instrument-making industry produces a vast quantity of general-purpose equipment each year (to the value of some 30–40 million rubles, including 8 million rubles on systems for the automation of routine analysis), it is not geared to small runs where fewer than 50 items are needed at a time. Moreover, many of the instruments produced are, Aleksandrov said, "frankly speaking, completely useless".

Aleksandrov drew particular attention to the failure, so far, of the Ministry of the Radio Industry to produce the special radar sets needed to monitor soil moisture and salinity. These devices, originally a spin-off from the Venus programme, have an important role to play in the union-wide Food Programme, Leonid Brezhnev's final bequest in 1982 to the Soviet Union. In spite of the efforts of the academy's Institute of Radio Engineering and Electronics in adapting the Venus radars to agricultural use, Soviet industry, Aleksandrov said, has not yet managed to produce the devices so urgently needed. **Vera Rich**

Italian research

Renewed discontent of staff

THE underpaid researchers at the Italian national research council (CNR), the largest state research organization in Italy, are getting impatient. Five months after the hopeful appointment as president of the Milan physiologist, Professor Luigi Rossi Bernardi, they are still awaiting action.

So at least says the Comitato Ricercatori, the researchers' committee which now claims 700 of the 2,500 CNR scientists as members. The Comitato appears to be pushing Rossi Bernardi faster than he wishes, or is able, to go. A Comitato spokesman complained last month of Rossi Bernardi's "refusal to make contact with us", and outlined old sores between the CNR staff and the universities, among which pay predominates. Even a top CNR researcher receives the equivalent of only a little over £9,000 a year, net of tax, compared with a top professor's £20,000. The most junior jobs in CNR and among university lecturers are paid about £6,500 and £9,000 respectively.

In Rome last week, Rossi Bernardi said he had now met the Comitato and "we agree on many points". Moreover, he said, "everything they say is obvious—what remains to be done is to do it". But some things are plainly easier to achieve than others.

According to the Comitato, one of the main CNR complaints apart from pay is the relative status of university and CNR staff. This will not be easy to change. The CNR advisory committees which plan the research programmes are dominated by 130 university professors against just 10 CNR researchers. Officially, CNR scientists are described as "collaborators", suggesting a role subservient to the universities. The Comitato also complains that the management of the 270 CNR research institutes and centres falls to university professors so that CNR scientists must "obey decisions without their being consulted". Yet according to statistics prepared by Rossi Bernardi himself, and published in the 1984 CNR report, CNR scientists published six times as many papers per head in 1983 as the average Italian scientist, who published only once in four years.

Thus Comitato members are pressing for at least a career structure comparable with that of the universities. The powerful trades unions, on the other hand, insist on a research "contract" structure for CNR under which researchers would be paid for work done.

The real problem, according to Rossi Bernardi, is to produce a new legal structure for CNR, in which promotion on merit is now virtually impossible. Salaries, taking a quarter of CNR's present £500 million annual budget, could hardly be increased without such a structure. So will a new law be possible? Rossi Bernardi says he is doing what he can, but that it is "a political problem".

Meanwhile, some reforms are under way. Thus Rossi Bernardi has proposed that CNR researchers should supplement their incomes by contracts and consultancies with industry. Then, acknowledging that CNR "cannot do everything", he says it should concentrate on areas of "strategic" importance.

Rossi Bernardi is also planning to concentrate CNR funds next year on CNR institutes whose performance after assessment is found creditable. He will pay out half the 1986 CNR research funds "on merit" he says, which will be a big change for CNR but one of which the Comitato Ricercatori should approve. Indeed, the Comitato says it will agree provided that merit is assessed "by the scientific community rather than by bureaucrats and union representatives", Comitato members "are ready to submit themselves to international competition" said a spokesman. **Robert Walgate**

Planetary missions

Soviet frankness cheers US

Washington

WHAT appears to be the first detailed account of the Soviet planetary exploration programme, including plans for an ambitious mission to Mars and its moons in 1988, has been provided by a delegation of Soviet space scientists in the United States last month. The delegation, led by Dr Valery Barsukov of the Vernadsky Institute, described a plan for soft-landing a small probe on the surface of Phobos, the larger of the two martian moons, and then making it "hop" to other points on the surface by the remote firing of small rockets.

Under the plan, two spacecraft will be launched in 1988, carrying solar-physics packages to study particles and fields in the ecliptic. After entering Mars orbit, one of the craft will assume a circular orbit matching that of Phobos, and manoeuvre to within 50 metres or so of its surface. In collaboration with French scientists, the Soviets are developing a 1-Joule laser that will vapourize and ionize surface material from Phobos, which will then be drawn through an on-board mass spectrometer for analysis.

The second craft will primarily be a back-up that may rendezvous with Demos, Mars's second moon, if the first attempt to land on Phobos is successful.

The delegation also described plans for a lunar polar orbiter to be launched in 1989 or 1990 so as to produce a detailed map of the Moon's geochemistry as well as for the possible mission to Venus in 1991 that would include dropping a lander on to an asteroid, possibly Vesta, on the way.

US scientists who heard the delegation's presentation, given at the Lunar and Planetary Science Conference at Johnson Space Center in Houston, say they were both surprised by the Soviets' frankness and impressed by the ambition of their plans.

Although Soviet scientists had previously outlined their planetary programme (most recently at a meeting arranged by the Planetary Society during last year's conference of COSPAR, the Committee on Space Research of the International Council of Scientific Unions, in Graz, Austria), last month's presentation filled in many gaps. It was welcomed as what seemed part of a move away from the secrecy and deliberate vagueness of previous Soviet announcements of future space missions.

Official contacts between the National Aeronautics and Space Administration (NASA) and Soviet space officials were ended in 1982, when the Reagan administration declined to renew a ten-year-old space cooperation agreement, citing the imposition of martial law in Poland. But in recent weeks, different administration officials have revived talk of cooperative space ventures, including a joint rescue training mission involving the US shuttle and the Soviet Salyut spacecraft and coordination of Mars exploration.

Last autumn, President Reagan signed a non-binding Senate resolution sponsored by Senator Spark Matsunaga (Democrat, Hawaii) calling for renewal of the cooperation agreement. Matsunaga has now filed another bill that would set up an "East–West" working group on Mars exploration.

During this hiatus, informal contacts between Soviet and US scientists have continued, typified by the arrangement by which US scientists built an instrument with NASA funds which is being carried on the Soviet Vega mission to Halley's comet (see *Nature* **312**, 3; 1985). There have been other informal approaches to US scientists qualified to supply instruments for the Soviet Venus-asteroid and other missions.

A more likely form of low-level cooperation, according to US specialists, is, however, data-sharing and join calibration of instruments on the Soviet Mars probe and the US Mars Geoscience Climatology Orbiter, due for launch in 1990. The Planetary Society is hoping that concrete plans to these ends will be agreed at the 1986 COSPAR meeting in Toulouse. **Stephen Budiansky**

Soviet espionage

Furore over French allegations

Le Monde, the Paris daily, has caused a furore by publishing what is alleged to be a secret report from the Soviet Ministry of the Aviation Industry on the "utilization of positive foreign experience"—in other words, industrial espionage. The disclosure, which coincided with the opening of high-level Franco-Soviet economic talks, caused the Soviet embassy in Paris to issue denials of this "flagrant disinformation" in even more vigorous terms than usual.

Le Monde claims that the disclosures were leaked to it by French intelligence and were the evidence on which President François Mitterrand expelled 47 Soviet diplomats two years ago. They include a description of the alleged inner workings of VPK, the Commission on Military Industries, which is said to issue "shopping lists" to Soviet representatives abroad, specifying what high-technology information the military establishment needs most urgently.

Claims that such lists exist have surfaced regularly in West Germany, and a recent Swedish survey of alleged Soviet industrial espionage by Charlie Nordblom (*Industri Spionage*, Timbor Förlag, 1984) specifically alleged that the State Committee for Science and Technology and the Academy of Sciences of the USSR are involved in compiling them.

The euphemistic Soviet description of the undertaking is not to be wondered at. At one time, the United States ran a Soviet-watching project, operating from the Weisbaden air force base in West Germany, known as STEP, the Science and Technology Exploitation Program.

The Soviet Union may have had advance warning of the impending disclosures in *Le Monde*. The president of the Academy of Sciences, Dr Anatolii P. Aleksandrov stressed to the general assembly of the academy last month that the Soviet Union intended to rely even less in future on imported know-how, while a few days before *Le Monde* published the documents, Radio Moscow broadcast an extensive interview with an "expert of the Soviet State Planning Committee", Dr Aleksei Petrov.

Petrov complained that the West had long suggested that Soviet technical progress depended on Western know-how, but "facts are facts and they show something quite different". During the past 20 years, Petrov said, the Soviet Union sold to the United States twice as many licenses as it had purchased, and almost two-thirds of Soviet imports consisted of raw materials.

Petrov's data, of course, referred to the civilian sector, while the *Le Monde* documents refer primarily to military aviation. To a Soviet planner, however, the distinction would probably seem artificial. The extremely low figures for military expenditure published in the Soviet budget (around 18,600 million rubles annually) make sense only if they refer to the replacement of existing military hardware, with military research and development absorbed by the civilian sector.

Vera Rich

Australian veterinary science

Lab. at last

Canberra

AFTER several years of to-ing and fro-ing about its value and safety, the $A160 million Australian National Animal Health Laboratory (ANAHL) was officially opened early this month. The ultra-secure laboratory at Geelong, 70 km south-west of Melbourne, was designed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) for rapid diagnosis of and research into exotic livestock diseases, especially foot and mouth disease virus (FMDV), not detected in Australia since 1872.

Over the years, the unfinished laboratory has been a repeated source of political contention. Two issues have arisen—the high cost of the facility, and the objections of many in Australia to the notion that FMDV might be imported, even if for research only, into a virus-free continent.

The laboratory is designed on the "box within a box" principle, in which the work area of highest security is at the centre of the building and is surrounded by a series of "boxes" of decreasing security classification.

For the present, ANAHL scientists are conducting investigations into endemic local diseases such as bovine leukosis and rhinotracheitis, and are attempting to clone the gene for the two outer protein coats of the local bluetongue virus, a serotype of which was isolated from insects collected in Northern Australia in 1977. In a few months, however, it is likely that work will begin on several exotic diseases including rabies, swine fever virus, Aujeszky's disease (pigs), exotic bluetongue, Newcastle disease (poultry) and vesicular diseases which could be mistaken for FMDV. Ultimately, permission may be granted for work on virulent exotics such as rinderpest or rift valley fever, but the importation of live FMDV has been shelved until 1987 at the earliest.

The laboratory will cost $A8 million a year to operate, but an outbreak of foot and mouth disease would result in the loss of an estimated $A3,000 million in export earnings.

Jeffrey Sellar

European research

Curien wants more

Strasbourg

PLANS for using science and technology to improve Europe's competitiveness with the United States were put forward in a speech to a gathering of geologists on 1 April by M. Herbert Curien, minister of research in the government of France. Curien's proposals, which have been hammered out over the past few months by groups of scientists and politicians, are to be presented to the Council of Europe early in May, at a meeting called for by M. François Mitterrand, the French president.

One of Curien's themes was European collaboration in space research and development, chiefly through the European Space Agency (ESA). The past twelve years of ESA's work, Curien said, had been creditable, but he urged last week that by the end of the century, Europe should be in a position not merely to build and launch satellites but to put men and women into space and to provide a full "customer service".

More generally, Curien said, Europe could respond to the apparent superiority of the United States in science and technology only if it were "united, ambitious and autonomous". Specifically, Curien lent his weight to proposals of the European Communities and the European Science Foundation (ESF) for research "networks". Since last September, some 50 proposals have been put forward and have now been whittled down to about a dozen.

The network plan is to involve groups of European research laboratories on common projects, much as several different groups of European geophysicists are now involved in the European Geotraverse project of deep seismic sounding (begun in 1983 by ESF). Last week, Curien referred to neurosciences and polar studies as potentially fruitful areas for collaboration on the same pattern.

Curien also wrung his hands last week over one of Europe's longstanding problems, the difficulty of encouraging mobility within the research community. He said that there were signs, in France, that mobility had recently declined. The difficulty, now as previously, is to devise appropriate incentives for the encouragement of mobility. There remain, said Curien, delays in arranging for the exchange of people, financial assistance to people on the move is lacking while there are even difficulties in transferring equipment across national frontiers. He urged that the impetus for the better coordination of the European research community should come from scientists themselves rather than from rule-bound institutions.

Peter Gambles

Brazilian energy

Physicists seek new nuclear policy

BRAZIL should limit its nuclear power programme to the one US (Westinghouse) reactor now operating, "study" the two West German (Kraftwerk-Union) reactors under construction and take more account of the environmental and social consequences of its grandiose hydroelectric schemes, according to a report prepared by Brazilian physicists and delivered to both houses of the Brazilian parliament late last month.

The physicists are still smarting from their anger at not being consulted when, in 1977, the previous military regime drew up plans with Kraftwerk-Union and others for an eight-reactor grid and a complete nuclear fuel cycle. They now say that the West German deal should effectively be cancelled.

The physicists are thus seizing the opportunity to shift the directions of Brazilian energy policy, now that Brazil has its first civilian elected president for some years, Señor Tancredo Neves. Neves made a cut-back in the nuclear programme part of his election platform—and the idea may have appealed to the electorate partly because the principal nuclear power construction site is at Angra dos Reis, on a beach between São Paolo and Rio de Janeiro in the middle of a popular resort region.

A response to the physicists' proposals has, however, been complicated by Neves's illness (he has had four intestinal operations in the past three weeks) which overtook the 75-year-old president-elect before he or his government could be sworn in. It is likely to be six months before Neves is fit to govern, and in the meanwhile few major policy changes are expected in Brazil.

Nevertheless, Neves has unofficially nominated to the new post of energy minister Dr José Israel Vargas, a physicist who specializes in energy matters and was prominent in Brazil's alcohol fuel (gasohol) programme. Vargas was involved in most of the previous military government's energy projects and is said to understand the economic pressures that led the military to commit Brazil to hydroelectric and nuclear power.

In the mid-1970s, the Brazilian economy in the south-east was growing at up to 12 per cent a year, and the national utility FURNAS sought an 11 per cent annual growth in production. Plans were made for 60 nuclear power plants to produce 75 GW by the year 2000, and construction began of one of the world's largest hydropower complexes on the border with Paraguay.

Brazilian physicists complain that the military's energy plans were made without any democratic consultation, and imply also that there was a hidden defence objective in the agreement with West Germany.

More sanguinely, a Brazilian government official said that "there was then great euphoria and trust in the future". Now the economy has slumped and atomic energy "doesn't make much sense".

The physicists' report, prepared at a meeting chaired by Dr Luigi Pinguelli Rosa of the University of Rio de Janeiro and attended by the president of the Brazilian physical society, Professor Fernando Souza Barros, and the chairman of São Paolo Electric Power Stations Limited, Professor José Goldenberg, recommends that the new government should:

- Cancel the agreement between the Brazilian nuclear power agency, Nuclearbras, and West Germany for "joint enterprises".
- Stop the construction of nuclear reactors outside Angra dos Reis, and "study" the continuation of construction of the two German reactors at that site.
- Question the effectiveness of the jet nozzle uranium enrichment supplied by West Germany.
- Restructure the Brazilian nuclear sector to make it more accountable and "civilian".
- Re-examine Brazil's nuclear future by open debate.
- "Give priority" to social and environmental considerations in the planning of future energy projects, particularly nuclear and hydroelectric stations.
- Take dam construction out of the exclusive hands of the electric utilities.
- And take account, in a democratic way, of the effects of dams on local populations and the local environment.

Robert Walgate

Gifts to universities

Threat from US tax reforms?

Washington

PRIVATE universities in the United States, which each year receive close to $3,000 million in charitable contributions from individuals, have been among the loudest to complain about US Treasury proposals for tax simplification that would reduce the allowable charitable deduction.

Congress has yet to take up tax reform seriously; but already an academic squall has broken out over the extent to which donors are truly influenced by tax considerations.

Under the Treasury proposal, charitable contributions would be deductible against income only to the extent that they exceed 2 per cent of the taxpayer's gross income. In addition, donations of non-cash items, such as real estate, art works and stocks, would receive far less favourable tax treatment. At present, the full appreciated value of such property can be claimed as a tax deduction, but under the proposal, the donor could deduct only the purchase price plus an allowance for inflation or the fair market value—whichever is lower.

According to a study made by Professor Charles Clotfelter of Duke University at the request of the Association of American Universities, overall contributions to universities by individuals would fall to 72 per cent of the present level if the Treasury proposal became law. Yet both the universities and those who challenge predictions such as Clotfelter's agree that the most damaging effect of the Treasury proposal would stem from the proposed lowering of the highest marginal tax rate from the current 50 per cent to 35 per cent. Instead of receiving 50 cents off of his tax bill for each dollar given, the wealthy taxpayer would receive only 35 cents off. This has placed the universities in an awkward spot: they can hardly be seen to lobby for higher tax rates.

The universities are thus taking the line that the damage inevitably done by lowering rates should at least not be made worse by further restricting charitable deductions. And they are especially concerned about the treatment of non-cash gifts, which account for 40 per cent of their contributions (and a proportionately higher share of contributions from the wealthiest donors). The universities estimate that the Treasury proposals would effectively double the "cost" to donors of such gifts.

A more fundamental question is whether the "cost" of giving matters to donors—particularly wealthy donors. Bruce Davie, chief tax economist of the House Ways and Means Committee, has recently identified an increase of donations by high-income taxpayers over the period 1975 to 1983, despite the reduction of the highest marginal rate from 70 per cent to 50 per cent in 1982.

Clotfelter, in an article to be published shortly, responds that the taxpayers in question had an 86 per cent increase in after-tax income, in constant dollars, during the same period but that their contributions went up by only 23 per cent in constant dollars. The universities are also attempting to prove their case by pointing to a sharp drop in contributions by taxpayers with gross incomes in excess of $1 million after the lower marginal rate went into effect in 1982; average contributions among this group fell from $205,000 to $145,000 in that one year.

Stephen Budiansky

When does life begin?

SIR — The widespread notion "that human life begins at conception" is not based on scientific evidence as J.R. Ling (*Nature* 24 January, p. 262) implied. It results from misleading oversimplifications and misconceptions about the biological basis of human individuality.

That our personal individuality develops after conception is conclusively shown by the natural occurrence of identical twins, triplets and so on. A fertilized egg is not yet "a potential human being" as J.R. Baker (*Nature*, 14 February, p. 524) puts it; left undisturbed, it may instead develop into several human beings, or even more commonly spontaneously abort without producing any. Because the timing of the split of the cell mass that produces identical twins is quite variable, no exact time can be given for the "start" of their individual human bodies — but it is always many days after conception.

If what we are interested in is not the start of individual body form, but the personality of individual humans (surely more important), this develops much later — only after the formation of the nervous system. Consciousness and a sense of self seem to develop only after birth and growing experience of the outside world.

If the vague term "human life" refers not to any of these features of the life of the individual but to human life in general, then this had no clear starting point at all in our gradual evolution from apes.

The notion of giving protection to all *potential* humans is an argument against contraception that would condemn us to gross overpopulation and misery. What we should protect vigorously are *actual* individuals possessing human feelings. The consequent legal and ethical necessity to choose a sensible dividing line at which such protection should be given must inevitably be somewhat arbitrary because of the gradualness of development. It is a fallacy to suppose that biology provides an easy answer to the problem.

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SIR — The leading article of 21 February (p. 612) asks ". . . who can object to the proposition that there can be no such thing as a human being without implantation?"

Let us examine how such a conclusion can be reached, beginning with some default assumptions about the meanings of the words used. Human life begins at conception (conception = the beginning of life), commonly taken as occurring at the moment of fertilization, the adjective "human" denoting the fact that both sperm and ovum are of human origin. Human life, in order to proceed, requires implantation; this also is not disputed. Having proceeded by means of this process, the life in question develops to a greater degree of complexity and, in due course, is (normally) born. At this point we are running into trouble because the same words ("human life") are used both before and after implantation. We must therefore define our terms carefully, as follows; the words "human being" must refer only to human life which has at some stage been carried in its "mother's" womb; from this your conclusion follows easily. But perhaps this is not good enough, for as is well known, a baby cannot become a living person unless cared for by adults (if we define our terms carefully), on whom he/she also depends for "emotional and physical security".

On the other hand, perhaps all this playing around with words is idle sophistry. One could as well say that human embryos are not persons because a person is a human being with full rights under law which a human embryo plainly does not possess. Obviously, it follows that not all human beings are persons.

What is to be gained by all this? The following; the public will become confused, Parliament will produce legislation riddled with loopholes, research will thereby go on and human embryos will continue to be subject to experiment and destruction.

Sir, you do science a disservice.

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Sequence data

SIR — A national service for the retrieval and analysis of nucleic acid and protein sequences is being supported by the Medical Research Council (MRC) and is available to academic workers in the United Kingdom. The service is based on 4 databases which are regularly updated : the EMBL Nucleic Acid Sequence Library (currently 2.1 million bases); the GenBank Nucleic Acid Sequence database (currently 3.3 million bases); the Protein Information Resource (0.5 million amino-acid residues) and the NEWAT Library of Doolittle[1]. Computer programs are available for database searches[2] and for the analysis of individual nucleic acid and protein sequences[3,4]. The Staden programs[5,6] for the experimental determination of DNA sequence have also been implemented. The service provides documentation of the databases and programs, help-files and facilities for communication within the user community[7]. There are currently some 50 scientists located at 15 sites throughout the United Kindom accessing the databases.

The service was established in collaboration with scientists from a number of departments at the University of Cambridge, particularly Dr M. Ashburner and Dr M. Bishop, and with the MRC Laboratory for Molecular Biology. The databases and programs are mounted on the IBM 3081 computer, maintained by the University of Cambridge Computer Service, and there are plans to mount them also on a VAX computer. The databases can be readily accessed through the Joint Academic Network (JANET) or through PSS from any location in the United Kingdom. Those interested should contact me or Dr Geoff Kneale at the address below (telephone: 0223 66499) for further information.

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1 Kneale, G G & Bishop, M.J. *Computer Applications in the Biosciences* 1, 11–17 (1985).
2 Bishop, M.J & Thompson, E *Nucleic Acids Res* 12, 5471–5474 (1984)
3 Staden, R *Nucleic Acids Res* 10, 2951 (1982)
4 Bishop, M.J. *Biochem. Soc. Trans* 12, 1008–1010 (1985)
5 Staden, R. *Nucleic Acids Res* 12, 499–503 (1984)
6 Staden, R. *Nucleic Acids Res* 10, 4731–4751 (1982)
7 Kneale, G G. & Kennard, O. *Biochem. Soc Trans* 12, 1011–1014 (1985)

Rings of light

SIR — Readers of *Nature* who look for intriguing optical phenomena in the world around them will have enjoyed Professor Thomas Gold's observation of puzzling relections in water[1]. He will, I am sure,regard it as a shared enthusiasm, and not a criticism, if I point out that others have observed and recorded these twists and loops too.

Minnaert's book[2] contains at section 14 ("Irregular reflection on slightly rippling water") a general bibliography stretching back to 1840, and he says of his own Fig. 17 "Remarkable is the appearance of closed coils of light . . ."

The most comprehensive discussion is perhaps in a book by Sir Montagu Pollock[3] published in 1903. His chapter on "Reflections in rippled water" outlines the geometry, though more particularly of the reflection of horizontal spars rather than of vertical masts. His book is illustrated with many plates by the Yorkshire marine photographers F.M. Sutcliffe and C.E. Wanless, who were fascinated by just such reflections of ships seen in the waters of Whitby and Scarborough harbours: Sutcliffe's Plate XXV is remarkably similar to Professor Gold's, and his Plate XXXV is appropriately titled by the artist "Rings".

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1 Gold, T. *Nature* 314, 12 (1985) and cover photograph, 7 March issue
2 Minnaert, M *Light and Colour in the Open Air* (Bell,London, 1940, reprinted by Dover, London)
3 Pollock, M *Light and Water, a Study of Reflexion and Colour in River, Lake and Sea*, (Bell, London, 1903)

Monitoring quantum jumps

A neat theoretical calculation shows how to monitor the state of a simple model atom without interfering with its quantum state. But Heisenberg's principle remains intact.

IF Heisenberg's uncertainty principle means anything at all, it must surely mean that it is impossible to keep a continuous record of the transition of a quantum system, say an atom, between two distinct quantum states. That is the general expectation. Suppose, for the sake of argument, that a particular atom may be found in one of only two states, a ground state and an excited state, and that some measurement is carried out to establish which state is occupied. The result is certain to be that the measurement will interfere with the system. Ordinarily the atom will be left in the condition that the measurement reveals, at least until something happens to shift it into the other state. So an ambition to monitor the state of the atom continuously would seem to conflict with these familiar expectations.

But, it seems the outlook may not be that unpromising. Richarch J. Cook of the United States Air Force Institute of Technology at Dayton, Ohio, and H.J. Kimble of the University of Texas at Austin, have devised what seems to be a neat way around the difficulty (*Phys. Rev. Lett.* **54**, 1023; 1985). Their proposal hangs on the techniques by means of which it has now become possible to confine single atoms (strictly, ions) in a small region of space by means of electromagnetic traps of various kinds (of which the Penning trap is the prototype). Spectroscopists nurse ambitions to use such traps as ways of carrying out spectroscopy with single atoms. Cook and Kimble's proposal for the direct observation of quantum jumps is one such application. To be fair, the authors do cautiously use the word "possibility" in the title of their paper. They go on to add that the effect which they predict has not yet been observed, "but probably will be in the near future".

This is how the proposed Cook/Kimble experiments would work. The first need is for an atom with two excited states which are connected to the ground state by transitions that are respectively strong and weak. Put one such atom into a Penning-like trap (which is naturally easier said than done) and illuminate it with a laser with a frequency which corresponds to the excitation energy between the ground state and the strongly coupled excited state, and measure the intensity of the fluorescence radiation of the same frequency which is given off by the single atom. What happens is merely that the atom is repeatedly excited (by the laser beam) and de-excited (by spontaneous radiation emission). If the ground and excited states are indeed strongly coupled, the rate of emission of fluorescence radiation can be as much as 100 million photons a second, well within the capacity of radiation detectors. And while, as Cook and Kimble say, the fluorescent signal will in reality be a succession of quantum events, there will be no difficulty in recording this as steady current in, say, a photomultiplier system. That is the first step.

Next, take a laser whose frequency corresponds to the energy difference between the ground state and the other excited state, that to which the ground state is only weakly coupled, and simultaneously illuminate the single atom with that as well. The predominant process will be the repeated excitation and de-excitation of the strongly coupled state, but occasionally the weak excitation will be excited instead. And when that happens, the electron whose oscillation into and out of the strongly coupled state is responsible for the main fluorescence signal will be out of action, marooned in the weakly coupled state instead. And since the coupling is weak, the main fluorescence signal will be turned off for the length of time the electron stays in the weakly coupled state. So the output from the photomultiplier circuit will consist of a series of signals corresponding to the full value of the strong fluorescence punctuated by gaps when the signal vanishes, and which correspond to the times when the weakly excited state is occupied. That, then, is the running record of when the weakly excited state is occupied. The signal from the photomultipliers will be, for all practical purposes, like telegraph signals in which the dots and dashes have random length. There will be no difficuly in interpreting the record with macroscopic measuring devices — the output signal can be drawn on a moving paper chart and interpreted by eye, if need be.

Cook and Kimble attribute the origin of their neat proposal to H. Dehmelt, who is said to have suggested a scheme rather like this in the context of the detection and measurement of weakly coupled optical transitions. Their own chief purpose is to provide a theoretical calculation of the details of the effects of competitive excitation within their three-level model atom on the fluorescent signal eventually produced, and in particular to calculate the statistical properties of the gaps in the fluorescence signal in terms of the parameters describing the two transitions.

The argument follows a now familiar line. The rate of excitation of each state is determined by the Einstein parameters from each transition, that which determines the rate of spontaneous emission (conventionally labelled A) and that which determines (with the intensity of the exciting radiation) the rate of excitation (labelled B). Cook and Kimble simplify the algebra somewhat by supposing that the light exciting the strongly coupled transition is intense enough to saturate the single atom (so that, if the weakly coupled excitation did not exist, the atom would spend exactly half of its time in the ground state and the excited states). The practical problem is merely to calculate the probability that the fluorescence signal due to the strong transition will persist for a length of time, say T. Inevitably, the results all depend on the Einstein parameters for each transition, but their practical value is that they involve the Einstein A parameter representing the rate of spontaneous de-excitation of the weakly-coupled state, a quantity that is not easily determined by conventional measurements.

This neat recipe for an experiment not yet carried out will obviously spur on the single-atom spectroscopists. On the assumption that the result is that which has now been predicted, the question obviously arises of the sense in which Heisenberg's uncertainty principle must be qualified. Fortunately, a little reflection will show that the question is a red herring. The underlying assumption in what Cook and Kimble have done is that the rate of transition between the ground state and the strongly coupled excited state is very much greater than the rate of transition to the weakly-coupled state. Moreover, there is no way in which this system could be used for arranging that the atom is excited one way or the other to order, for the state of the atom at any time is determined by strictly random processes.

So despite this apparent assault, the uncertainty principle remains intact. What does, however, emerge from this neat piece of experimental design is a vivid illustration of the interesting things that will be possible when single-atom spectroscopy moves into the laboratory, which should not be much delayed.

John Maddox

Cellular immunology

Immunological help at last

from Jonathan C. Howard

AN opportunity to celebrate the end of nearly twenty years of ignorance about an essential immunological relationship is provided by the paper of Lanzavecchicia on page 537 of this issue[1]. The relationship is that which forms between T cells, B cells and antigen molecules when T cells provide help to B cells during the induction of antibody formation. The conceptual significance for cellular immunology of what has now been accomplished is difficult to overstate since T-cell help provides the paradigm for all specific regulation of the immune response. Perhaps the best evidence that something important has happened recently is that the two best up-to-date textbooks[2,3] contain no hint of the elegant and, I will risk saying, certainly correct model that I am about to describe.

In Burnet's clonal selection theory, each clone of lymphocytes was autonomous, distinguished from others by the specificity of its cell-surface receptor for antigen. In theory, the selecting power of antigen alone was sufficient to define lymphocyte clones destined for antibody synthesis. However, the 'autonomous' model of antibody induction had to be abandoned as a result of pressure from two directions. First, Burnet's 'lymphocytes' were shown to consist of two developmentally independent and functionally distinct lineages of cells. In particular, thymus-derived (T) lymphocytes never turned into antibody-producing cells, whereas marrow-derived (B) lymphocytes did so, but only if T lymphocytes were also present. T-helper cells therefore somehow helped B cells to make antibody. Second, it became clear that T cells made an antigen-specific contribution to B-cell induction from studies on the structural properties required for antigens to be immunogenic. For an antibody to be raised in animals against any defined small determinant or hapten it had to be attached to a carrier molecule that was an immunogen in its own right. In a series of influential experiments, Mitchison[4] showed that the requirement for T-helper cell in the induction of antibody responses against hapten-carrier conjugates resulted from the obligatory recognition by T cells of antigenic determinants on the carrier. In addition, hapten and carrier had to be linked on the same molecule. To accomodate these facts, Mitchison proposed the antigen-bridge model for T-cell help (Fig. 1), in which the antigen is bound through a haptenic determinant to the B-cell receptor, which is immunoglobulin (Ig) and is simultaneously bound through a carrier determinant to the T-cell receptor. For over a decade this model has provided the only accessible mental image of antigen-specific T-B collaboration.

It was 'MHC restriction' that ultimately destroyed the antigen-bridge model. It is an operating principle of the immune system that T cells can recognize and be activated by antigen only when the antigen is associated with a molecule of the major histocompatibility complex (MHC) on a cell surface. It was shown first by Katz *et al.*[5] (in a remarkable experiment that anticipated by quite some time the conceptual advances needed to explain it fully), and subsequently by others, that antigen-specific T-B interactions are MHC restricted in just this sense. To visualize the structural basis of such interactions, it was supposed that the antigen specific T-cell receptor forms an antigen bridge with the B cell, as in the Mitchison model, while a hypothetical second T-cell receptor binds independently to MHC molecules expressed

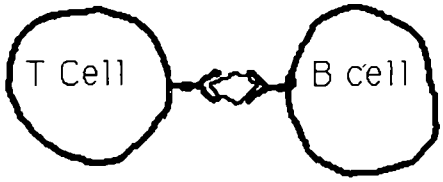
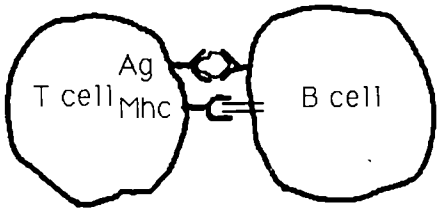


Fig. 1 Antigen-bridge model. The T cell binds to a carrier determinant on the antigen, the B cell binds to a distinct haptenic determinant.

Fig. 2 Modified antigen-bridge model, incorporating MHC restriction. The T cell is given a second receptor (Mhc) with which to bind B-cell Ia molecules. The T-cell receptor for the antigen (Ag) is the same as in Fig. 1. Sometimes the two independent binding sites for Ia and antigen are shown as attached to the T cell via a single stem.



on the B-cell surface. It is an interesting commentary on the importance of the visual imagination that this dual-receptor image (Fig.2) should have persisted at a time of general acceptance that MHC-restricted T-cell recognition of antigen is mediated by a single receptor with a single combining site[6]. It has, however, been difficult to see how, with a single receptor, a T-helper cell could first be activated by antigen associated with an MHC molecule on a presenting cell, and then deliver help in an MHC-restricted manner to a B cell bearing the antigen in its Ig receptor. For a single receptor, how could the structure antigen + MHC be equivalent to the structure antigen + Ig + MHC?

The resolution of this paradox began when Benacerraf[7] pointed to strong circumstantial evidence that protein carriers were not normally in their native configuration when they were recognized in an MHC-restricted manner by T-helper cells. Both he and Unanue[8] suggested — and it was later demonstrated[9] — that native antigen is taken up by phagocytic cells, partially degraded and then returned to the cell surface as peptide fragments in association with MHC class II molecules (H-2 Ia in the mouse, HLA-DR in the human). This complex then stimulates specific T-helper cells. Similar peptide/Ia associations could exist on B cells and be recognized by active T-helper cells. Furthermore, the Ig receptor of B cells should concentrate specific antigen on some B cells rather than others.

The clearest early formulation of the new model that I know is from Uhr and his colleagues[10]. If T-helper cells recognize Ia-associated fragments of carrier antigens presented on macrophage surfaces, they wrote in 1980, "this presumption implies that the B cell can only be specifically helped by a T-helper cell if a similar antigenic fragment is presented by the B cell. Thus, we could hypothesize that the B cell can pinocytose and degrade antigen like the macrophage. The only difference would be that Ig receptors for specific native antigen equip the corresponding B-cell clone with a means for capturing antigen which is then processed and presented by the B-cells' Ia antigens to the T-helper cell."

This new model of antigen-specific help, represented in Fig.3, has been substantiated by some particularly striking and elegant experiments, of which Lanzavecchia's, on page 537, represent the culmination. It has been crucial to show that the B-cell Ig receptor functions as an antigen-concentrating device, rather than an antigen-presenting device, for specific B cells. Grey and colleagues were the first to show that molecules captured by B-cell Ig receptors can be efficiently presented to T cells[11]. They went on to show that B-cell lymphomas can internalize and process non-specifically-bound antigen, and re-present it in the form of peptide fragments to MHC-restricted T-helper cells[12].

These important experiments fell short of demonstrating that specific antigens captured by the B-cell Ig receptor normally are processed and re-presented in that way. Last year, Rock *et al.*[13] found that TNP-specific B cells, purified from an immune spleen, could present a TNP-carrier conjugate to carrier-specific helper T cells; a strikingly low concentration of conjugate was required compared with that required for presentation by unselected normal B cells. These results showed, for the first time, that specific antigens captured by the Ig receptor are ultimately presented very efficiently to T cells: what they did not prove is that antigens thus captured are processed before presentation.

Lanzavecchia has finally settled the issue using antigen-specific T-cell and B-cell clones from the same individual and prepared against the same antigen, tetanus

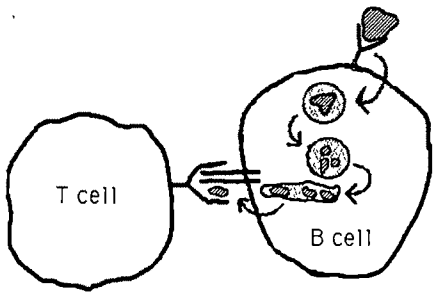


Fig. 3 The new model. The T cell only has one receptor, which recognizes a complex of processed antigen and Ia molecule on the B-cell surface. Antigen processing is initiated by attachment of native antigen to the Ig receptor.

toxoid (TT). The B-cell clones, transformed by Epstein-Barr virus, possess Ig receptors with affinity for TT and present TT in an MHC-restricted manner to TT-specific T-cell clones. In terms of free antigen concentration, TT capture by the B-cell Ig receptor is approximately four orders of magnitude more efficient than non-specific uptake of TT. Lanzavecchia shows that the Ig receptor is involved only with antigen capture, and not directly with antigen presentation, by separating the two events in time. B cells are pulsed briefly with antigen, washed and then allowed to present the antigen to T cells: while the uptake of antigen during pulsing can be prevented by an antibody to Ig, the same reagent has no effect on the ability of pulsed B cells to present antigen to T cells.

As the new model requires, uptake of the TT is not enough for its presentation to T-helper cells; the antigen must also be internalized and processed. Thus, uptake is followed by a stage which is sensitive to the lysosomotropic agent, chloroquine, already known to prevent antigen processing[14]. After this stage, antigen-pulsed B cells can be fixed with gluteraldehyde and still present antigen to T cells. By analogy with other work[12], the fixative-resistant state occurs when processed derivatives of the antigen have been returned to the cell surface and have become associated with class II MHC molecules.

Although the haptenic determinant plays no necessary role at the time when help is delivered to the B cell, and although antigens are digested into peptides before presentation, the requirement of hapten-carrier linkage is satisfied by the new model. Only B cells with a receptor for determinants on the native molecule can present carrier determinants efficiently to T cells.

As long as antigen-bridge models were current it was assumed that if the B cell recognized one part of the molecule, then the T cell must recognize another, so that both could bind together to form the antigen bridge. In the new model this condition disappears completely. B cells of any specificity that can bind and internalize an antigen can presumably receive help from any T cell that can recognize the processed form of the molecule.

Like Grey and Rock, Lanzavecchia assays the response of T cells to antigen presented by B cells. For help, it is clearly the response of the B cells to T-cell recognition that counts. Lanzavecchia has recently shown (personal communication) that EBV-transformed B-cell clones respond to antigen-specific interaction with T cells. In the same antigen dose range that stimulates T cells, specific T-cell clones induce what seems to be terminal differentiation in the EBV clones; cell division is arrested and immunoglobulin release increases.

The quantitative implications of the new findings give as much pleasure as the qualitative. Both Rock *et al.* and Lanzavecchia show that B cells can accumulate and present immunologically significant antigen with an external concentration in the range of 10^{-12} M, which is low enough to be physiologically meaningful. In Lanzavecchia's case, the affinity of the B-cell receptor for TT is about 10^{-8} 1 mol^{-1}. At an external concentration of 10^{-12} M, the mean occupancy of the B-cell receptor by a univalent ligand such as TT would be about 0.01 per cent, or about 10 molecules per B cell with 10^5 Ig receptors. Surely this is not enough to process and present to T cells. An attractive solution to this paradox is that continuous receptor recycling allows specific B cells to accumulate processed material. Clearly, this option, which I call 'pumping', is not available to B cells in an antigen-bridge model. In confirmation of the pumping hypothesis, Lanzavecchia has now shown (personal communication) that it takes longer for B cells to acquire the ability to stimulate T cells, the lower the external concentration of antigen.

Lanzavecchia's experiments are a considerable achievement. The clarity and elegance that come from studying the interaction between two antigen-specific clones are bought at a high price in technical skill. Nor are there many experiments in cellular immunology of such great pedagogic value as these, which display so accurately the formalism that they are intended to represent. It is a special bonus that these experiments are with human, not rodent, cells.

I do not doubt that the new model is essentially correct. There are however, unresolved problems of considerable interest. Does the binding of antigen to the B-cell receptor deliver a necessary differentiative signal to the B cell, or does the B-cell receptor function only to capture antigen? How is the recognition of processed antigen-MHC complexes transduced into a differentiative signal for the B cell (or, indeed, for the T cell) and what is the causal relationship between this signal and isotype-switching? To what extent does B-cell proliferation and differentiation depend on the availability of non-specific (in an immunological sense) growth factors of either T-cell or B-cell origin? These are important questions but none, surely, so central to immunology as the one that has finally been settled. □

1. Lanzavecchia, A *Nature* **314**, 537 (1985)
2. Paul W.E. (Ed) *Fundamental Immunology* (Raven, New York, 1984).
3 Hood, L E , Weissman, I L , Wilson, J H & Wood W.B *Immunology* 2nd edn (Benjamin-Cummings, 1984).
4 Mitchison, N A *Eur. J Immun* **1**, 18 (1971).
5 Katz, D.H., Hamaoka, T , Dorf, M.E., Maurer, P H & Benacerraf, B *J exp Med.* **138**, 734 (1973).
5. Matzinger, P *Nature* **292**, 497 (1981).
7 Benacerraf, B *J. Immun* **120**, 1809 (1978).
8 Unanue, E *Adv Immun* **31**, 1 (1981)
9 Unanue, E & Ziegler, K. *J Immun.* **127**, 1869 (1981)
10 Lipscomb, M.F , Lyons, C F., Ben-Sasson, S Z , Tucker, T.F & Uhr, J.W. in *Macrophage Regulation of Immunity* (eds Unanue, E. & Rosenthal, A) (Academic, London, 1980)
11 Chesnut R.W. & Grey, H M *J. Immun* **126**, 1075 (1984).
12 Shimonkievitz R., Kappler, J W., Marrrack, P. & Grey, H M *J exp Med* **158**, 303 (1983).
13 Rock, K.L., Benacerraf, B & Abbas, A K *J exp. Med* **160**, 1102 (1984).
14 Ziegler, K. & Unanue, E *Proc natn. Acad Sci. U.S A* **79**, 175 (1982).

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Gamma-ray astronomy

How much H_2 in the Milky Way?

from Leo Blitz

ON PAGE 511 of this issue Bhat *et al.* deliver a new salvo in a battle that has been raging among astrophysicists since 1980 over how much molecular hydrogen there is in the Milky Way. The crux of the issue is how to convert observed line strengths of the CO molecule into absolute quantities of molecular hydrogen; Bhat *et al.* argue for a lower quantity than any yet published.

The debate has been fierce, emotional and at times has degenerated into *ad hominem* attacks. But why so much bile over such a seemingly esoteric quantity? The reason is that the real issues are much larger astrophysical questions such as: how, when and why do stars form in a spiral galaxy; why do spirals look different from one another; and how do these spiral galaxies evolve?

The source of the controversy dates to the years around 1970 when millimetre-wavelength radio astronomy was in its infancy. A plethora of molecules was discovered in the interstellar medium, including water, ammonia, formaldehyde, carbon monoxide, ethyl alcohol and a number of molecules not observed terrestrially. It was quickly demonstrated that excitation of the key species implied collisions by neutral hydrogen molecules at high density (by interstellar standards) and at a temperature as low as about 10 K. The problem is that when the gas is cold, molecular hydrogen does not emit an observable quantity of radiation and therefore cannot be directly observed. In-

stead, its presence has to be inferred from trace constituents with abundances at most 10^{-4} that of H_2. The most widely used tracer is CO because it is the easiest to observe, but estimating H_2 masses from CO observations is like estimating the weight of a dog from the number of hairs on its tail. To make matters worse, CO is an optically thick molecule; the strength of its radio-spectral lines is more closely related to the temperature of the gas cloud than to how many molecules are radiating.

Two significant discoveries in the mid-1970s emphasize the importance of obtaining accurate H_2 masses from the CO observations. The first is the ubiquity of CO in the plane of the Milky Way, implying that a significant fraction, perhaps most, of the interstellar gas is H_2 and not atomic hydrogen (H I), which previously had been thought to be the reservoir of the star-forming gas. The second is that essentially all present-day star formation takes place in giant molecular clouds, which have masses of about 10^5 solar mases and are composed almost entirely of H_2 (but detected by radio observations of CO). Therefore, the question of star formation on a galactic scale was reduced to the question of how giant molecular clouds form. Do they, for example, condense from the atomic gas, or do they form from the collisional accretion of small molecular clouds without the mediation of an atomic phase? Important clues were to be found in the relative amounts of H_2 and H I. (Ultimately the answers can be applied to studies of other spiral galaxies. The evolution of the disk of a galaxy is essentially its star-formation history, which in turn is largely the history of the giant molecular clouds.) The results of the first CO surveys of the galactic plane implied that between the galactic centre and the position of the Sun, which is about half-way out, the interstellar gas is overwhelmingly molecular. One study suggested it contained ten times as much H_2 as H I, which in turn implied that atomic gas is largely irrelevant to the formation of giant molecular clouds, but these conclusions were quickly challenged by several groups who argued that the molecular phases are about equal in abundance inside the solar distance. In that case, instabilities in the atomic component are likely to play an important role in the formation of the giant molecular clouds.

The different estimations have been largely the result of using different ratios to convert CO line strengths to H_2 column densities, and also to the importance of temperature and heavy-element abundance variations in the Milky Way. New light has been shed on this subject by gamma-ray astronomy. It was established in the 1970s that diffuse high-energy gamma-rays (> 50 MeV) come primarily from the interaction of cosmic rays with the interstellar gas. If the gamma-ray emissivity of the gas can be determined, the number of hydrogen nuclei in a column along a given line of sight can be obtained from measurments of the gamma-ray intensity. The contribution to this intensity from atomic hydrogen is known from 21-cm line observations; the remaining contribution must therefore be from molecular hydrogen (corrections due to helium and other trace constituents are straightforward). In principle, this approach is the most accurate way to determine the H_2 from CO, because the gammas rays directly trace the dominant component of the gas — hydrogen — independently of excitation and abundance variations in the CO.

Interpretation of the gamma-ray results has not been without its problems, however. Initially, the results from the US SAS-2 satellite and the European COS-B satellite were not in agreement — a large and difficult to determine instrumental background for COS-B seems to have been the culprit — and other technical problems with the data analysis have only recently been solved. Nevertheless, the quantity of molecular hydrogen in the Milky Way reported by the Durham group on page 511 of this issue is still half that derived by the Caravane collaboration, which launched COS-B. The groups agree, however, that the gamma-ray data unequivocally imply that the H_2 does not dominate the H I inside the solar distance.

More H_2 would simply produce more gamma rays than are observed. The work of the Durham group implies that nowhere in the Milky Way does the H_2 surface density dominate that of the H I. In the published work of the Caravane collaboration, H_2 may dominate weakly within a small range of distance from the galactic centre. The residual difference between the two groups probably results from systematic uncertainties that increase the formal erros assigned to the data by Bhat *et al.* A new sophisticated analysis by the Caravane collaboration using the most extensive available data sets is forthcoming and should shed additional light on this nagging astrophysical controversy. □

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100 years ago

THE way in which a bird builds its nest, seemingly without instruction, has been brought forward as a proof of blind instinct governing it in its task. A remarkable instance, however, of a changed mode of nest-building has been brought to my notice by Mr W. Burton. His brother took to New Zealand some young chaffinches. Some of the birds have built a nest, and the structure shows very little of that neatness of fabrication for which the bird is noted in England. The cup of the nest is small, loosely put together, and the walls of the structure are prolonged for about eighteen inches, and hang loosely down the side of the supporting branch. Clearly these New Zealand chaffinches were at a loss for design when fabricating their nest.

From *Nature* 31 533, 9 April 1885.

Evolution

Interpretations of mass extinction

from Michael J. Benton

IT IS becoming clear that the history of life has been punctuated by a series of events during which, typically, an apparently random selection of organisms died out. These mass extinction events can be seen to have 're-set' the evolutionary clock: they wiped out whole families or orders of plants and animals, irrespective of the darwinian fitness of the individual organisms or of the species involved, and created opportunities for the survivors to radiate out into the 'empty' ecospace that was left. The recent suggestion that mass extinctions might be cyclical in occurrence is important because it raises the possibility of identifying one kind of explanation for all mass extinctions through time, whether or not the events have a regular periodicity and whether the ultimate causes are terrestrial or extra-terrestrial. These ideas have important implications for evolutionary biology and for historical geology.

The most important recent impetus to the discussion of the causes of mass extinctions has been the suggestion of Raup and Sepkoski[1], from their analysis of the fossil record of marine animals, of a regular periodicity of about 26 Myr to mass extinctions over the past 250 Myr. Hallam[2] has pointed out how uncertainties in dating of mass extinctions, especially before the late Cretaceous, weaken the claim of regular periodicity, but the suggestion remains a very important one and is having a considerable influence on the ways in which we may interpret a cyclical pattern of mass extinctions.

Four interpretations of the cyclicity are possible and worth considering. The first two are deterministic and the other two stochastic viewpoints: (1) each extinction event was triggered by the same external impulse, acting directly; (2) the cyclical pattern of extinction events correlates with some physical phenomenon that follows the same cycles (for example, fluctuations in temperature or sea level), and these are controlled by a third external variable; (3) the events are caused by a variety of factors that relate to the internal dynamics of the biological system; (4) the apparent cyclicity is an artefact of the patchiness of the fossil record.

The first interpretation is favoured by Raup and Sepkoski and their followers, who suggest an extraterrestrial cause for the periodic mass extinctions. Three main schools of thought have sprung up. The

first is that a hypothetical companion star of the Sun (called Nemesis) disturbs the Oort cloud and sends a shower of comets hurtling towards Earth every 30 Myr[3,4]. The second is that the whole of the Solar System oscillates through the galactic plane with a semi-period of 30 Myr and periodically scatters Oort cloud comets into the terrestrial zone[5-7]. The third idea is that the unknown Planet X, located beyond the orbit of Pluto, follows an eccentric path which causes it to disturb a comet belt beyond Neptune and precipitate a shower of comets, some of which would eventually reach the Earth, every 28 Myr[8]. These astronomical theories are still highly speculative and controversial (see *News and Views* 7 March p.17).

The second interpretation, that cycles of mass extinction correlate with cycles in terrestrial phenomena, has been the generally held view for some time. Fischer suggested that the Earth has passed through cycles of different orders of magnitude[9,10], the largest of which each lasted about 300 Myr and began with rapid convection in the mantle. This led to the break-up of Pangaea-like supercontinents, elevated sea levels and marine transgressions, intense vulcanism, release of CO_2 to the atmosphere and the development of a greenhouse climate, resulting in worldwide warm conditions and warm seas. Such episodes occurred from late Cambrian to late Devonian and early Jurassic to late Eocene.

The second phase of each 300-Myr supercycle was characterized by low mantle convection, continental accretion, low sea level and regression, reduced atmospheric CO_2 and the development of an icehouse state. This gave high latitudinal climatic gradients, cold dry polar regions and cold oxygenated ocean waters. These conditions characterized the latest Precambrian to early Cambrian, the late Palaeozoic to Triassic and the latter half of the Cenozoic, in which we still live. The crossovers from greenhouse to icehouse correspond to mass extinctions in the late Cambrian (500 Myr), the late Devonian (355 Myr), the late Triassic (192 Myr) and the late Eocene (40 Myr). These crossovers are about 150-Myr apart, giving a span of 300 Myr for the full cycle. This model does not explain the terminal Permian and the terminal Cretaceous extinction events.

Fischer also described two classes of shorter-range cycles in tectonic activity and world climate[9,11], one set occuring every 30 Myr and the other consisting of smaller climatic cycles in the range 20,000-400,000 years, which are induced by orbital pertubations. The 30-Myr cycles are reflected in temperature fluctuations, variations in carbon isotope ratios and in the fossil record of planktonic and nektonic taxa.

Newell[12,13] and Hallam[14,15] have argued that sea-level changes are the most important prime cause of mass extinctions, thus laying the main emphasis on one segment of the Fischer theory. Sea-level changes have exhibited a cyclicity on several scales through the Phanerozoic and many of the well-known mass extinctions correlate well with worldwide episodes of regression. In general terms this usually coincides with a reduction in the area of diversity of continental shelf habitats, and in some cases (for example, the end-Pliensbachian, end-Cenomanian and possibly the end-Triassic, late Devonian and end-Ordovician events) with periods of oceanic anoxia, as recorded by extensive black shale deposits.

The other-kind of terrestrial explanation, which modifies part of Fischer's thesis and relegates sea-level changes to a minor role, emphasizes changes in temperature. This has been championed recently by Stanley[16-18]. He notes evidence of global episodes of cooling that coincide with mass extinctions in the late Eocene and the Plio-Pleistocene and hints at evidence for temperature changes linked to earlier mass extinction events (it becomes harder to pin down temperature changes the further back in time one goes). Stanley supports this thesis with the fact that mass extinctions have frequently been concentrated in the tropics, which became a refrigerated trap into which high-altitude forms migrated and were then wiped out. He also argues that the long periods of time that characterize most mass extinctions accord more with a gradual change in temperature than with a catastrophic change brought about by an exterrestrial agent.

If, however, cyclicities in geological processes should turn out to have regular periodicities, an extraterrestrial cause will have to be considered. Has the time come with the discovery by Rampino and Stothers[19] of two dominant periodicities, of about 33 and 260 Myr, in a time-series analysis of various geological phenomena (sea-level fluctuations, sea-floor spreading discontinuities, tectonic activity, geomagnetic reversals)? These correspond to the two major orders of cyclicity identified by Fischer, Rampino and Stothers suggest they were caused by periodic comet impacts. The issue at stake is clearly the evidence for regular periodicity in geological processes, which has been questioned (see *News and Views*, 7 March p.17).

There are important philosophical consequences if we accept some or all of these aspects of regular cyclicity in mantle, plate-tectonic, eustatic, atmospheric and climatic processes and their concomitant effects on the history of life. Periodicities of these kinds would form a part of Charles Lyell's interpretation of Uniformitarianism which has generally been abandoned[20]. Lyell believed that there were constant changes on the Earth, but that these cycled endlessly and with no direction. He would not accept the idea of progressionism in the history of life, believing instead that any life form could exist at any time if the conditions were right: thus he expected to find Silurian mammals, or dinosaurs, at some future time. This anti-progressionist stance was ignored or abandoned by most other scientists during Lyell's lifetime, and the idea of uniformity of conditions has also not found favour. Now it must be reconsidered if we accept that most major processes on the Earth have followed, and continue to follow, long-term cyclical patterns.

The third interpretation of cyclical mass extinctions — that they arise from the internal dynamics of the biological system — has recently received support from Kitchell and Pena[21]. Attempting to fit different time-series models to the extinction curve of Raup and Sepkoski, they find that the best statistical fit is a stochastic time-series model that shows 'pseudocycles' every 31 Myr. This model takes account of the observed spacing and the relative magnitude of each peak of mass extinction. Kitchell and Pena conclude that each extinction event should be investigated on its merits; it may be incorrect to seek an overall controlling pattern or an ultimate cause that explains all mass extinctions.

The fourth explanation, which is that the apparent regular pattern is an artefact, has also received support. Re-analysing Raup and Sepkoski's data, Hoffman and Ghiold find that they correspond just as well to a neutral model, in which origination and extinction rates vary independently and randomly through time, as they do to the deterministic periodic model[22]. Episodes of high origination and high extinction rates usually correspond with highly fossilferous geological units. The apparent periodicity of 26 Myr in mass extinctions could be an artefact of the average length of stratigraphic stages, the basic time units used in the calculations.

We are left with all four explanations for mass extinction cycles. Arguments for and against each view will continue. But more detailed analyses of the fossil data, and refinements in dating events in the geological past, will be necessary before we can decide which view is correct. □

1. Raup, D M & Sepkoski, J.J , Jr *Proc natn Acad Sci U S A* **81**, 801 (1984)
2. Hallam, A *Nature News and Views* **308**, 686 (1984)
3. Whitmire, D.P & Jackson, A A *Nature* **308**, 713 (1984)
4. Davis, M , Hut, P & Muller, R A *Nature* **308**, 715 (1984)
5. Rampino, M.R & Stothers, R B *Nature* **308**, 709 (1984)
6. Schwartz, R D & James, P B *Nature* **308**, 712 (1984)
7. Clube, S V M. & Napier, W M *Nature* **311**, 635 (1984)
8. Whitmire, D P. & Matese, J.J *Nature* **313**, 36 (1985)
9. Fischer, A G in *Biotic Crises in Ecological and Evolutionary Time* (ed Nitecki, M.H) 103 (Academic, New York, 1981)
10. Fischer, A G in *Catastrophes and Earth History* (eds Berggren, W.A & Van Couvering, J A.) 129 (Princeton Univ., Princeton, N J., 1984)
11. Fischer, A G. & Arthur, M A *Spec Publ Soc econ Paleontol Miner.* **25**, 18 (1977)
12. Newell, N D *Spec Pap geol Soc. Am* **89**, 63 (1967)
13. Newell, N D in *Catastrophes and Earth History* (eds Berggren, W A and Van Couvering, J.A.) 115 (Princeton Univ., Princeton, N J , 1984)
14. Hallam, A *Nature* **269**, 769 (1977)
15. Hallam, A *A Rev Earth planet Sci* **12**, 205 (1984)
16. Stanley, S M *Geology* **12**, 205 (1984)
17. Stanley, S M *Scient Am.* **250**, 46 (1984)
18. Stanley, S M in *Mass Extinctions* (ed Nitecki, M H) (Univ. Chicago Press, 1984).
19. Rampino, M R & Stothers, R B *Science* **226**, 1427 (1984)
20. Gould, S.J in *Catastrophes and Earth History* (eds Berggren, W A and Van Couvering, J.A) 9 (Princeton Univ , Princeton, N.J , 1984).
21. Kitchell, J A & Pena, D *Science* **226**, 689 (1984)
22. Hoffman, A & Ghiold, J *Geol Mag* **122**, 1 (1985)

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Anthropology

Improved timing of hominoid evolution with a DNA clock

from Peter Andrews

OF THE many contributions in the past year towards the taxonomic applications of protein, amino-acid and DNA analyses, it is worth singling out the approach of DNA-DNA hybridization, which has been claimed by C.G. Sibley and J.E. Ahlquist (*J. molec. Evol.* **20**, 2; 1984) to provide new insights into the evolution of the hominoid primates. How well does this claim stand up to examination?

Molecular approaches to taxonomic studies were initiated by Morris Goodman, who compared the equivalent protein in different species by immunological techniques. This was followed by Sarich's calculations of protein 'distances' from microcomplement fixation data. Others have calculated allelic differences measured by electrophoresis. What these methods have in common is that they produce overall distance measures from the study of proteins, and if they are compared with morphological analyses they are seen to be akin to the numerical taxonomy and multivariate analyses that were performed in the 1950s and 1960s.

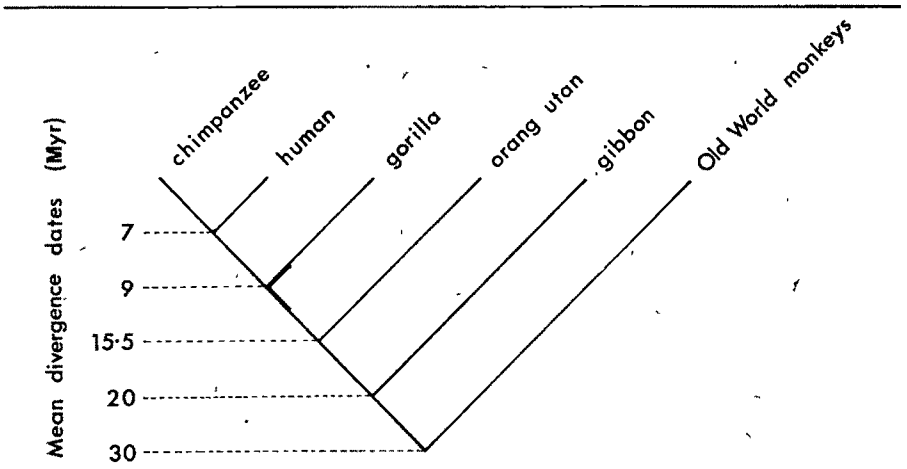
Two claims are made for these protein analyses. One is that genetic similarity between taxa can be identified by minimizing the genetic distance between them, and from this the relationships and evolutionary branching patterns of the taxa can be inferred. The other is that the rates of change of the proteins are sufficiently regular for the genetic distances to be applied as a clock. As to the first claim, while it seems likely that the inferred branching patterns are usually correct, it is not possible to test for correctness. This is because there is no way of knowing what properties of the proteins are contributing to the distances measured — the same problem encountered with morphological distances in numerical taxonomy and multivariate analyses for very much the same reasons. The necessary data to overcome this fundamental objection only becomes available when proteins are sequenced (for instance, Goodman *et al. Nature* **303**, 546; 1983).

There are also problems with the use of genetic distances as a clock. The clock hypothesis assumes correctness of branching patterns, and goes on to estimate relative times between branching points based on distances observed. The relative times are translated into absolute times by calibration, usually from the fossil record. Taking the example of the Hominoidea, the zoological group to which man and the apes belong, the branching pattern of the various species of hominoid is established according to the protein data, relative times of divergence between man, chimpanzee, gorilla, orang utan and gibbon are calculated on the basis of the distances between the species and absolute times of divergence are calculated by calibrating one of the relative times with a 'known' divergence date from the fossil record (usually the separation of hominoids from the Old World monkeys).

Two problems affect the accuracy of the protein clock. First, the fossil calibration date may be wrong because it is based on the earliest appearance in the fossil record of a species belonging to a particular lineage. It gives only a minimum date for the origin of the lineage, and the 'earliest appearance' is something that is always subject to change from new evidence. Second, the rates of change of the proteins being analysed may change along or between lineages; Goodman and others have shown that, particularly for the Hominoidea, the rate of change in a number of proteins is not constant. The protein clock must therefore be said both to be uncertainly calibrated and to keep irregular time.

Much of what has been said so far applies equally to DNA-DNA hybridization. In this technique, fragments of disassociated single-stranded radioactively tagged DNA from one species are reassociated with single strands from other species; the greater the base-pair similarity between the strands of DNA, the greater the degree of reassociation between them and the greater the force needed subsequently to disassociate them again. Measurement of this force serves as a new measure of overall genetic similarity between the species tested. This similarity is reasonably assumed to be homologous (although inevitably some will be due to homoplasy) but no distinction is made between homologies that are shared uniquely and those that are present in some distant common ancestor. But almost all of the similarities in DNA between hominoid species are likely to be the result of shared mammalian or primate inheritance, and only a few will be unique to the hominoids. Therefore, although DNA-DNA hybridization is based on the whole genome, only a very small part of the genome is relevant to hominoid relationships. How to distinguish this part from the rest of the DNA molecule is not stated by Sibley and Ahlquist, but if it is not distinguished how is it possible to test the claims made for it?

This question is not so intractable as it might seem. First of all, it is justly claimed that when the whole genome is tested the numbers of changes and homologies are so great that discrepancies are minimized for more distantly related taxa. But this might not apply to fine resolution. For example, DNA hybridization is claimed to link man more closely with the chimpanzee than the gorilla — a relationship that is not resolved by any of the protein distance methods. In fact, the distances given (with standard deviations) are 1.8–1.9 ± 0.2 for man/chimpanzee, 2.4 ± 0.2 for man/gorilla and 2.1–2.3 ± 0.2 for chimpanzee/gorilla. This degree of resolution is not impressive, given the size of the standard deviations and that only a small proportion of the genome contains relevant homologies, and I would not consider the question of man's closest living relative to be solved by these data.



The major limitation of the hybridization data is overcome by comparing DNA sequences instead, because both the number and kind of differences between species is unambiguous when sequences are compared. Thus, although the hybridization results seem merely to be confirmed by sequences of a short section of the mitochondrial DNA of the hominoids (Brown, W.M. et al. *J. molec. Evol.* **18**, 225; 1984 and Hasegawa, M. *et al. Proc. Japan Acad. Sci.* **60**, 95; 1984), the sequence data impart a level of confidence that the branching order, shown in the figure, is correct.

Having accepted the branching order, we can return to the DNA hydridization results and consider the value of the DNA clock that was derived from them. Sibley and Ahlquist find a much greater regularity of DNA change than has been demonstrated for protein change, for which there is

evidence of slowing down or speeding up in some cases. To calibrate the clock from the fossil record, Sibley and Ahlquist used two possible dates — 13 and 16 million years — for the divergence of the orang utan. The correct date is probably somewhere in between so that, by taking a range, the other branching points shown on the figure can be estimated.

The conclusion seems to be that DNA hybridization provides a useful insight into evolutionary events, at least for the hominoids. The resolution of branching patterns is perhaps not quite as great at has been claimed and, since it is inherently untestable, needs to be tested by sequencing data. More sequencing data will be needed to confirm the identification of man's nearest living relative. The DNA clock seems to keep very good time, in contrast to the protein clock, and gives a divergence time between man and the African apes of between 7 and 9 million years ago. In this connection, the recent description of a new hominoid from the Samburu Hills of Kenya (Ishida H. *et al. Af. Study Monog.* 2, 73; 1984) is of particular interest. It is placed at about 9 million years ago (the heavy black line on the figure) and is said to be either an immediate ancester of the African apes and man or an African ape with some similarity to the gorilla. □

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Helmholtz's choice

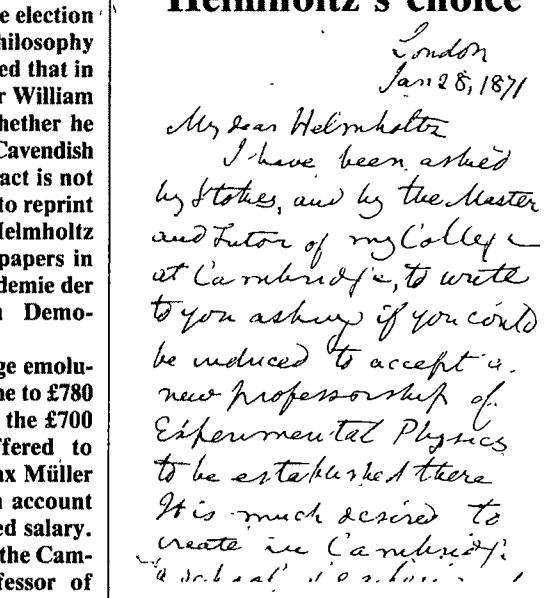
In my note "Opportunity Lost in 1865?" (*Nature* 306, 313; 1984) about the election of the Chair of Experimental Philosophy at Oxford in the 1860s, I remarked that in 1871 Helmholtz was asked by Sir William Thomson (later Lord Kelvin) whether he would accept the newly founded Cavendish Chair in Cambridge. Since this fact is not widely known, it seems justified to reprint in full Thompson's letter to Helmholtz which is among the Helmholtz papers in The Central Archives of the Akademie der Wissenschaften der Deutschen Demokratischen Republik in Berlin.

The combined university/college emoluments suggested to Helmholtz came to £780 to £800, only slightly more than the £700 which Oxford would have offered to Helmholtz six years earlier if Max Müller had not vetoed the invitation on account of the inadequacy of the proposed salary.

Helmholtz, who at the time of the Cambridge approach was still Professor of Physiology in Heidelberg, declined the offer since in June 1870 he accepted the Chair of Physics at the University of Berlin and was due to take up his appointment in the spring of 1871. There seems to be no record of Helmholtz's answer to Thomson. There is nothing in the Kelvin collection at the Cambridge University Library, but there is a letter from Helmholtz's wife to her mother dated 9 February 1871 (quoted in *Anna von Helmholtz, Ein Lebensbild in Briefen* Verag für Kulturpolitik, Berlin, 1929) in which she wrote that "Hermann has just received an enquiry from Sir William Thomson whether he would be inclined to accept a Professorship in Experimental Physics in Cambridge, under very attractive conditions — however it is to be Berlin" ("*Allein num ist doch Berlin die Losung*"). Then, in a letter dated 13 February 1871 the chairman of the Appointments Committee wrote to Maxwell asking him to stand as a candidate and expressing the hope that his election would be secured. It seems that these matters were dealt with most expeditiously a hundred years ago and that the postal services were gratifyingly swift.

I thank Dr Christa Kirsten, Director of The Central Archives of the Akademie der Wissenschafften der DDR, for the permission to reproduce this letter and Mr J. Deakin, Secretary of the Cavendish Laboratory, Cambridge for the information about the invitation to Maxwell.

N.Kurti, University of Oxford



My dear Helmholtz

I have been asked by Stokes, and by the Master and Tutor of my College at Cambridge, to write to you asking if you could be induced to accept a new professorship of Experimental Physics to be established there. It is much desired to create in Cambridge a school of experimental science, not merely by a system of lectures with experimental illustrations but by a physical laboratory in which students under direction of the professor and his assistant or assistants, would perform experiments, and the professor would have all facilities attainable, for making experimental investigations.

The Duke of Devonshire has already given £6000 for building the laboratory and making a commencement of providing it with instruments. I think it may be confidently expected that funds will not be wanting for carrying out this part of the place well. The University proposes to give £500 per annum to the professor, whose income will also be increased somewhat by fees from students. But if you were accepting the professorship it is I may say quite certain that you would also be appointed at least to a fellowship in St Peter's College which would bring an additional income of £250 or £300.

Thus I believe the income to be relied on would be not less than £800. But I think it probable that a praelector-fellowship of Trinity College would be offered to you, although on this point I speak merely from my own judgement. The duties of the praelector-fellowship, a new institution in Trinity College (of which one in physiology has been given to Dr Michael Foster and it was intended there should be also one in experimental physics) would be fulfilled by giving facilities to members of Trinity College for attending the professorial lectures: that is to say if the *university professor* is appointed to the praelectorship. Otherwise the praelector would lecture on physics in Trinity College simply. The income of the praelectorship would be about £600 so that the professor if appointed to the praelectorship would have £1100 per annum.

I know that the question of amounts of income is very far from the first in respect to the inducements which might possibly cause you to think of accepting such a proposal: and I am quite prepared to learn that you are "absolute for" Berlin. Still it is a question that must be weighed before you could decide to accept, and therefore I have told you what I can regarding it.

If you could at all entertain the idea of coming to this country, I think that the situation at Cambridge would present many advantages. It would certainly be a most interesting field, in respect to our profession, as the desire for physical science is growing stronger and stronger in the University, and the force of public opinion is steadily advancing in support of it, and to stimulate it where stimulus is needed.

The duties of *lecturing* would occupy only 20 weeks out of the year, and all the remainder of the time would be available for experimental or mathematical investigation, and scientific writing. Will you give me a line at the earliest, to say if you think you could be induced to accept or if on the contrary you decide at once against it. I need not say that it would be a great gratification and advantage to English scientific men to have you among us instead of merely having very rare opportunities of seeing you, and that I myself would consider the difference of distances from Glasgow to Cambridge and Berlin a great gain.

I write necessarily in haste, and can only add that, irrespectively of this question, I wished to write to you asking for news of yourself and of your family, and particularly regarding how you have been affected by this terrible war. I hope very much that you have not now cause for anxiety in respect to your son or other relations.

I am in London on account of a Committee invited to advise the Admiralty on Designs for Ships of War, especially in respect to stability, in consequence of the loss of the "Captain" which was upset with 500 men in the Bay of Biscay last September. When you write, address The College, Glasgow, as I am in London only for a few days each fortnight.

Believe me

Yours always truly

William Thomson

Yellow rain and the bee

SIR — Criticism of the bee faeces theory by Rosen *et al.*[1] is misleading and inaccurate. Their assignment of the burden of proof to US government sceptics is misplaced.

The authors assert that "heavy" invasion of substrate is a prerequisite for high toxin levels and that they never observed any "fluffy" mycelium in their samples. In fact, the relationship between mycelial mass and toxin production by *Fusarium* spp. is extremely variable, ranging well over three orders of magnitude. Further, the authors neglect to mention that they did not microscopically examine their samples. While at the University of Wisconsin, I examined bee faeces samples from Thailand. No mycelial growth was obvious but *Fusarium* spp. were present at counts as high as 1,000 colony forming units per milligram. At least one of these strains is capable of producing greater than 1,000 p.p.m. zearalenone under laboratory conditions. It is curious that the authors choose to subject their samples to highly sophisticated GC-MS analysis without also determining the mycoflora present by dilution plating and/or microscopy.

Rosen *et al.* claim that Type A and Type B trichothecenes are "never (except in one case)" found together. This "lone exception" is purported to be a corn sample collected in France. As at least one of the authors should know, this is incorrect[2].

In their final paragraph, Rosen *et al.* state "We are amazed that the proponents of this theory have not yet presented one iota of evidence that trichothecenes can be produced naturally . . .". When one considers the resources available to the US government's laboratories and their intense efforts to identify additional positive samples, it is truly "amazing" that none have been found. More importantly, it is astonishing that the authors imply that the burden of proof should lay with the sceptics. Meselson and others have merely advanced an alternative explanation that is consistent with all physical evidence. In contrast, the US State Department has termed its evidence as "unequivocal" and the so-called "smoking gun".

This "conclusive" evidence has been the basis of serious allegations of Soviet treaty violations and has directly affected such issues as the binary weapons programme and disarmament negotiations. Under those circumstances, who has the burden of proof?

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1 Rosen, J D , Cohen, H , Mirocha, C.J & Schiefer, H B *Nature* 313, 271 (1985)
2 Mirocha, C J., Schauerhamer, B , Christensen, C M & Koddedahl, T *Appl. Envir Microbiol* 38, 557-558, (1979)

Fivefold symmetry in boron and borides

SIR — A recent News and Views article presented an interesting discussion of fivefold crystal symmetries[1], in particular relating to the recent experiments on Mn-Al alloys[2]. I would like to draw attention to the fact that the fivefold symmetry is also a characteristic feature of the crystalline boron (beta-rhombohedral modification) as well as some borides (such as AlB_{12}). The crystal lattice of β-boron is formed by the repetitive pattern of icosahedrons with the unit cell having 105 non-equivalent atoms. This is by itself rather peculiar for an elemental material. This fact, strikingly little known outside the boron specialists, leads to a number of non-trivial consequences. One example is the possibility of hopping conductivity in an intrinsic (and not doped) semiconductor[3]. Therefore, the naturally occurring fivefold symmetry has another area of manifestation, besides the Mn-Al alloys mentioned.

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1. Maddox, J *Nature* 313, 263 (1985)
2 Shechtman, D *et al* 53, 1951 (1984)
3 Berezin, A A *J chem. Phys* 80, 1241 (1984).

King Midas and the Red Queen

SIR — Benton's commentary[1] on recent tests of the Red Queen hypothesis draws attention to the difficulty of distinguishing changes in the biotic environment from those in the abiotic one, over geological time. The hypothesis demands that evolution is driven by an *antagonistic* biota. However, *mutualistic* interactions among species cause the biotic environment to improve rather than to deteriorate[2], and the need for continual change just 'to keep in the same place' is lost. Thus, an alternative way of testing the hypothesis is to compare the rates of evolution of taxa in antagonistic environments with those found in mutualistic ones, specifically in associations where one species lives inside the cells of another. We suggest that the homeostatic intracellular environment experienced by mutualistic endosymbionts is buffered from antagonists while, at the same time, subject to much the same major abiotic changes (such as climatic perturbations) as the environment of their hosts.

Nature has been kind enough to provide many such evolutionary experiments, including mutualistic symbioses as diverse as algae within coelenterates (for example, corals), and bacteria within plants (for example leguminous and non-leguminous nodules, see Table 1 of ref. 2). A striking feature of these and other mutualistic associations is that the symbionts which live intracellularly for a prolonged period are represented by a much smaller number of taxa than their external partners[2]. We argue that this supports the view that biotic interactions do play an important part in evolutionary processes[2,3].

For those inclined to regal metaphors, we would contrast the Red Queen of deteriorating, antagonistic environments, with King Midas's golden touch of species in improving, mutualistic environments, although the outcome is evidently more stable for mutualistic species than it was for the king himself[3].

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1 Benton, M.J *Nature* 313, 734 (1985)
2 Law, R. & Lewis, D.H. *Biol. J Linn Soc.* 20, 249 (1983)
3 Law, R in *The Biology of Mutualism: Ecology and Evolution* (ed Boucher, D H) (Croom-Helm, London, in the press)

Biological role of IgG hinge region

SIR — We feel compelled to point out that an article correlating IgG antibody flexibility and complement fixation by the classical pathway[1], while an important piece of work, is a regrettable example of authors failing to acknowledge the intellectual and experimental contributions of earlier workers. Surely the findings of Oi *et al.* must be considered a quantitative refinement of principles laid out originally by us in a series of papers over the past decade (for example refs 2–6). The following[5], for example, is unambiguous:

> (These) studies ... highlight the crucial role played by the hinge region and its constituent disulfides in allowing IgG to perform its biological effector functions. An essential feature of this role is to limit segmental flexibility; yet this flexibility seems to be important for optimal antigen binding. Thus, the hinge serves to balance the antigen-dependent and antigen-independent flexibility requirements of the molecule.

It is surprising that the editorial review practices of *Nature* can be so accommodating to what we believe an imbalanced presentation.

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1 Oi, V.T *et al Nature* 307, 136–140 (1984)
2 Isenman, D E , Dorrington, K.J & Painter, R H *J Immun* 114, 1726–1729 (1975)
3 Romans, D G., Tilley, C A , Crookston, M C , Falk, R E & Dorrington, K.J *Proc. natn Acad. Sci* 74, 2531–2535 (1977).
4 Romans, D G , Tilley, C A & Dorrington, K.J *Molec. Immun.* 16, 859–879 (1979)
5 Klein, M *et al. Proc natn Acad Sci U S A* 78, 524–528 (1981)
6 Dorrington, K J & Klein, M H *Molec Immun.* 19, 1215–1221 (1982).

The World Ocean Circulation Experiment

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The international oceanographic community is preparing for an ambitious five-year experiment. Starting in 1990, it will provide the first comprehensive global survey of physical properties of the oceans. The resulting data set will be used to test computer models of the ocean circulation needed for predicting decadal climate change. The results will also benefit research in marine chemistry, biology and geology. This review looks at the climatalogical problems that motivated the project and discusses new observing techniques, from space and in situ, *which make it timely to embark on this major step in physical exploration of the ocean.*

THE World Ocean Circulation Experiment (WOCE) is being planned to survey the global distributions of ocean variables with a view to greatly improving estimates of the circulation of heat, water and chemicals around the world ocean, and their exchange with the atmosphere. Such experiments become necessary when the existing database is inadequate for a particular purpose; a new data set stimulates new thinking and reveals deficiencies in old ideas and models. A recent example was the Global Weather Experiment (GWE) in 1979, which has had a major impact on improving models used in weather forecasting[1]. The aim of WOCE is to collect a data set that will do the same for oceanic circulation. It will provide the first comprehensive global perspective of the ocean as an element in the planetary climate system. The experiment will open a new chapter in the history of ocean exploration, as the *Meteor* Atlantic expedition did in 1925-27, the *Discovery* Southern Ocean expeditions in the 1930s, the International Geophysical Year (IGY) Atlantic expedition in 1957 and the International Indian Ocean expedition in 1966 (ref. 2).

This article opens with a brief survey of the climatological problems that motivate the WOCE, then considers the present state of ocean circulation modelling, and surveys the data available to test them. The urgent need for a new global data set becomes evident. Finally, the aims, options, strategy and design of the experiment intended to meet that need are discussed.

Decadal climate change

There are many potential applications of a detailed understanding of the global circulation of the oceans, but the driving force for attempting a WOCE is the recognition that predicting decadal climate change will depend on accurate calculation of changes in the large-scale flow of heat, freshwater and chemicals in the oceans. The global pattern of climate on the continents is strongly influenced by those fluxes[3]. Estimates based on the difference between the radiation budget at the top of the atmosphere and the fluxes carried by the atmosphere[4] have revealed that the meridional heat fluxes in the ocean and the atmosphere both lie in the range 0.1–1 PW (10^{15} W), with the ocean contributing more in the tropics and the atmosphere more at higher latitudes (Fig. 1). Attempts are being made to extend the calculations with a view to discovering how much the flow in each ocean basin contributes to this zonally averaged heat flux[5–7]. This indirect approach has been stimulated by concern that calculations based on maps of surface energy fluxes may be unreliable because of errors in the bulk formulae used to calculate the fluxes from ship meteorological observations and uneven distribution of and errors in the observations. Global maps of heat and freshwater circulation based on the best available estimates are characterized by large flows between the basins (Fig. 2)[8]; the mean energy and water supply to the atmosphere in the Atlantic depend in part on solar heating and rainfall in the Pacific. Attempts have been made to calculate the fluxes more directly from hydrographic data at a few sites[9] (Fig. 3); the method gives values in the same range (0.1–1 PW) but differing significantly in detail. The great width of the Pacific Ocean presents special problems, and there is disagreement about the direction of the fluxes in some regions.

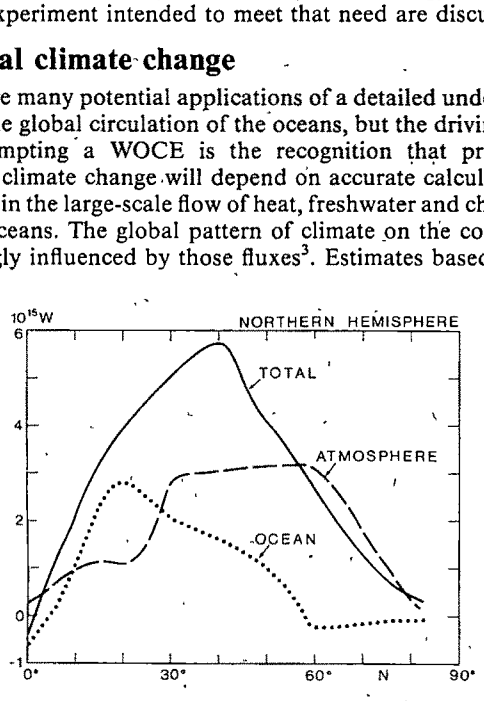


Fig. 1 The meridional heat transport carried by the ocean and the atmosphere[4]

The bulk formula method is suspect because it gives unrealistic budgets for the Mediterranean and Red Sea[10]; the direct method can be used reliably only at locations where the flux calculation is insensitive to the unknown barotropic component of the geostrophic current; the residual method is suspect because calculations of the atmospheric flux divergences give unrealistic values over the continents. The discrepancies between the three methods are quite modest by comparison with those in other aspects of planetary physics, and might be thought of as presenting no urgent challenge. But we are not concerned here with the energy balance of some distant planet. We are discussing the energy and water budgets which govern the climate of Earth[11]. The impact of climate change on mankind provides the motivation for predicting future changes in climate. Climate prediction provides challenging specifications for the accuracies to which we must measure ocean fluxes.

Consider the North Atlantic. The incoming heat flux (1.2 PW) is lost to the atmosphere at a basin-average rate of 50 W m^{-2}. The flow is unevenly distributed, but errors in the bulk method prevent us from mapping the distribution more accurately than ±50 W m^{-2}. The global patterns of surface heat and water fluxes are not constant; they exhibit a broad spectrum of variability. There has long been speculation[12] that such oceanic changes cause some of the observed variations in climate over the continents, but because of the deplorable inaccuracy of the methods used to measure the surface fluxes, there is usually no hope of deducing the changes from past records. Exceptions are sites such as the Eastern Equatorial Pacific, where interannual variation exceeds 50 W m^{-2} (ref. 13).

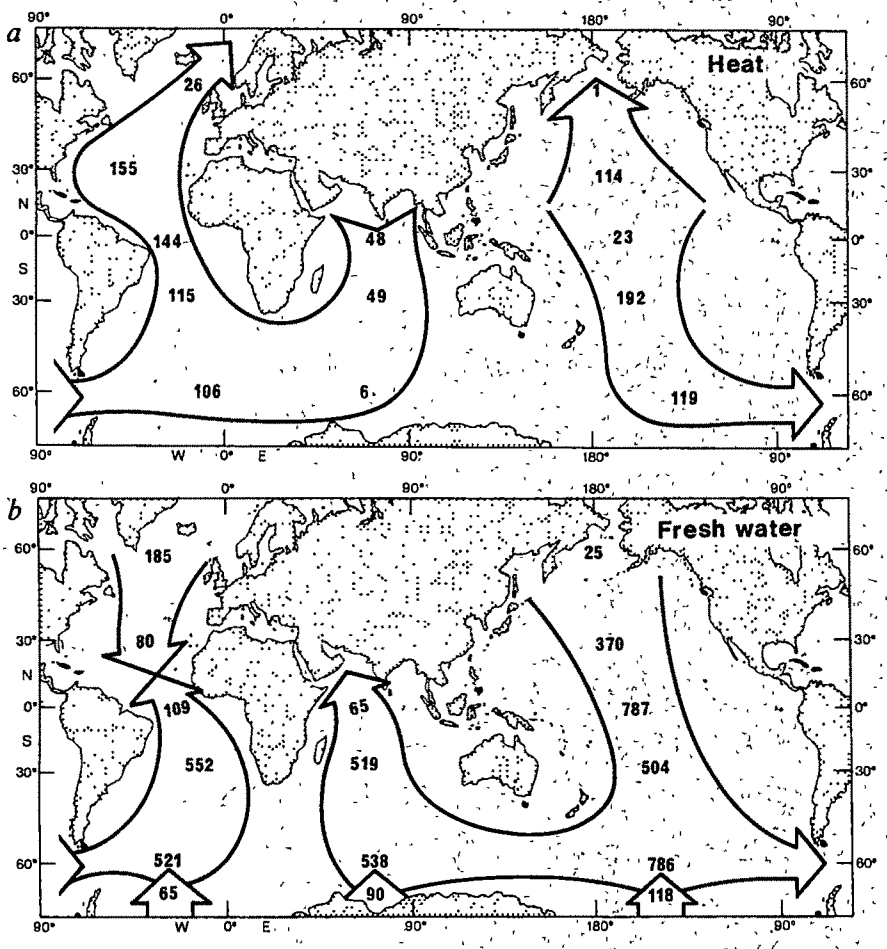


Fig. 2 Global circulations of heat (*a*, 0.01 PW) and fresh water (*b*, 10^3 tonnes s^{-1}) based on surface fluxes estimated from ship weather observations[8].

Even quite small changes in the surface fluxes averaged over large areas can have a large impact on climate if they persist for decades. They can be provoked by changes in the solar constant or in the atmospheric concentrations of dust or gases. The palaeoclimatological record derived from polar ice cores contains evidence of such changes[14]. The forcing might involve quite a small deviation in the surface radiation flux; for example, the natural doubling of atmospheric P_{CO_2} during the past 15,000 years changed the surface infrared flux by about 10 W m^{-2} (ref. 15). Sensitivity tests with atmospheric climate models have shown that important consequences can result from such forcing[16].

When we consider the possibility of forecasting decadal climate change, we become concerned with the processes inside the ocean (circulation and mixing) that provide the link between changes in surface forcing and the resulting changes in energy, water and gas fluxes to the atmosphere. Here we concentrate on the energy flux, although for some purposes the change in gas flux (especially CO_2) is also important. The scientific problem is to calculate the thermal response of the ocean to an externally induced change in surface radiation balance. The forcing changes the heat content of the ocean, leading to a change in the surface temperature and consequently in the rate of oceanic cooling to the atmosphere. Given a step change in the radiation input, the oceanic temperature will relax to a new equilibrium value, for which the globally averaged surface energy flux budget is again balanced. The new balance will involve a different partition between the components that make up the total cooling to the atmosphere; long-wave radiation, conduction (sensible heat flux) and evaporation (latent heat flux). The climate is sensitive to that partition. An increase of solar constant or of P_{CO_2} will lead to increased evaporation balanced by greater precipitation, some of which will fall on the continents.

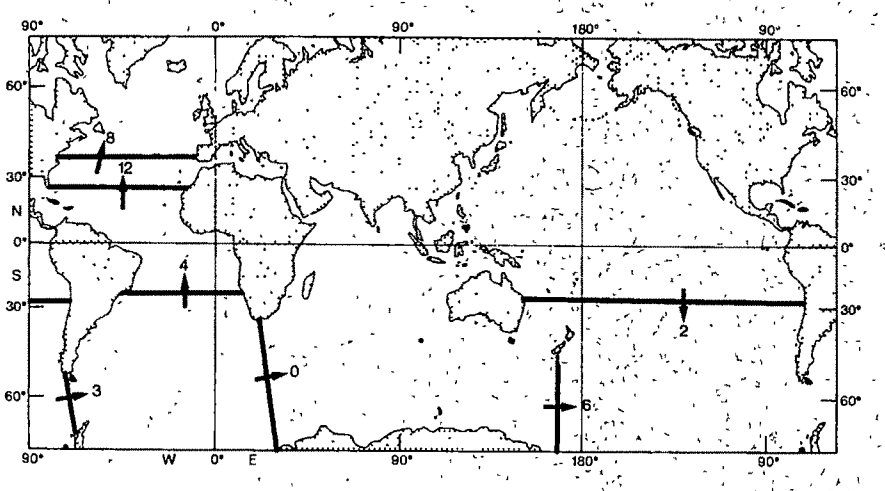


Fig. 3 Direct measurements of heat flow (0.1 PW) in the ocean based on hydrographic sections and other oceanographic measurements[9].

Decadal changes of ocean heat content are not confined to the surface mixed layer (mean depth 70 m), but are ventilated by deep winter convection and by Ekman pumping to depths of the order of 1 km (ref. 17). The involvement of a greater mass of water prolongs the relaxation time, giving ocean currents scope to redistribute the change of heat content over great distances. As a result, the equilibrium change in surface cooling and evaporation may be distributed quite differently from their mean patterns and from the distribution of surface forcing. Furthermore, the transient change may have different characteristics in different parts of the world, and the annual mean response may involve different seasonal changes at different locations.

To summarize, the global patterns of seasonal and annual climate—in particular rainfall—will change over decades in response to radiative forcing of the ocean by natural variations of solar constant and atmospheric dust and gas concentrations. These lead to changes in ocean heat content. There is no prospect of predicting decadal climate changes until it is possible accurately to model the action of oceanic circulation and mixing in redistributing the decadal heat content anomalies, as an essential prerequisite to calculating the changes in surface fluxes of energy, water and gases to the atmosphere[18].

A computer model designed to predict decadal climate change must, therefore, include the ocean circulation and mixing, as well as the atmospheric circulation and physics of clouds, radiation and precipitation. No such comprehensive model exists at present, although several groups are working towards that end[19]. The atmospheric component of such a model is well advanced, but the oceanic component lags behind. Ultimately the two components must be integrated because of feedback between the atmospheric climate and the oceanic circulation. It will not be satisfactory to treat the ocean currents as a stationary feature of a changing climate, but that refinement is of secondary importance compared with the urgent task of creating a model that can at least simulate the circulation of the ocean forced by a steady atmospheric climate. The availability of such a model would revolutionize our ability to diagnose the role of the ocean in our present climate, and would act as a springboard for developing the coupled atmosphere-ocean models needed to predict decadal changes of climate.

The strategy for oceanographers contributing to the problem of predicting decadal climate change is to develop a model that accurately describes the circulation of heat, fresh water and selected dissolved chemicals (notably those involved in the carbon cycle), using a description of the present seasonal cycle of atmospheric forcing; to test this model against a description of the circulations derived from observations; and progressively to improve the model until it predicts the fluxes of energy, water and gases (notably CO_2) into the atmosphere with errors significantly smaller than the observed decadal fluctuations.

Modelling the ocean circulation

The aim of WOCE is to collect a data set that will test and stimulate the development of models of ocean circulation needed for predicting decadal climate change. The class of model to be considered here is sometimes called 'prognostic', but it is not yet used in a forecasting mode, as that would require coupling to an atmospheric model. It is used in a diagnostic mode, being designed to calculate the seasonally varying distributions of currents, temperature, salinity and chemical concentrations around the world ocean, given the surface fluxes of momentum and vorticity, radiation, heat, water and gases as functions of season and geographical distribution. One of the triumphs of oceanography in the past decade has been the successful development of such a model at Princeton[20]. Starting with a uniform motionless ocean, the model has been integrated for the equivalent of 1,000 years with prescribed surface boundary conditions to give realistic patterns of surface geostrophic flow (Fig. 4).

The output of such a model integration can be used as a synthetic data base for the world ocean, with the desirable

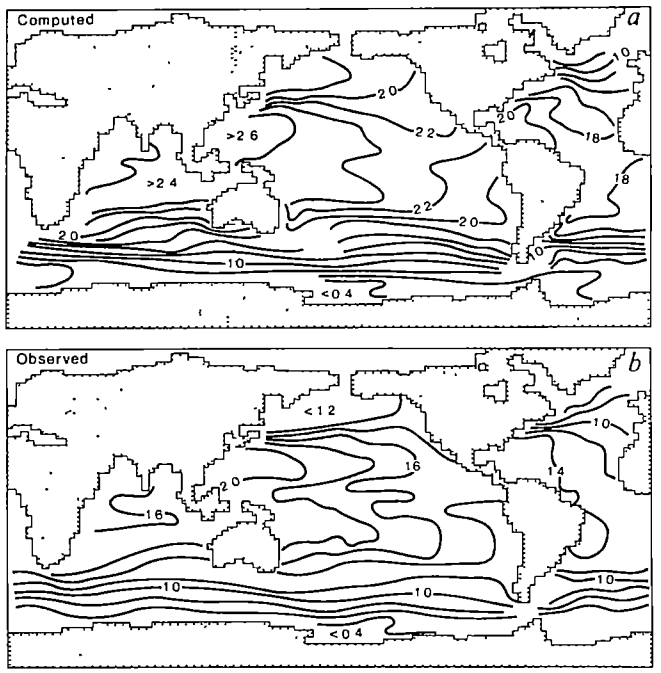


Fig. 4 The distribution of surface elevation of the ocean relative to the geoid (*a*) from the Princeton model (*b*) deduced from the observed density distribution using an assumption that the velocity is zero at 2,000 m (ref. 20). The surface geostrophic flow goes along the isolines, with a speed given by the horizontal gradient of surface elevation (that is, hydrostatic pressure)

attribute that there are none of the data-sparse regions that plague analysis of real data. For example, it is possible to calculate the fluxes of heat and water through trans-ocean sections[21]. The results agree remarkably with those derived from analysis of observations (notably across the Atlantic at 23 °N), giving us confidence in the model. Although encouraging, are the predictions sufficiently accurate to meet the needs of climate forecasting? And if not, can we identify and correct the sources of error in the way that meteorologists have systematically improved models used in weather forecasting? We do not have an adequate data base to answer such questions fully; that is why oceanographers need a WOCE, just as meteorologists need the GWE. We can, of course, spot obvious differences between the distributions of temperature and salinity predicted by the model and those printed in oceanographic atlases[22], but we cannot say whether differences are significant for prediction of decadal climate. However, most oceanographers are concerned about the difference in structure of the permanent thermocline (Fig. 5), since that is likely to be symptomatic of an error in the rate of ventilation of heat from the surface into the interior of the ocean, a process of central importance for decadal climate change[23,24].

One reason that the world ocean model gives a false thermocline structure may be that, to run it on the Princeton computer (one of the most powerful in the world), the spectrum of motion has had to be truncated at 250 km. The energetic eddies which constitute the oceanic equivalent of weather systems were not resolved; their effects on the velocity, temperature, salinity and chemical distributions had to be parameterized in the model equations by Boussinesq eddy transport coefficients (Fig. 6). Furthermore, the large truncation scale meant that rather large values had to be used for those coefficients; it was not possible to simulate the observed regional variation of eddy kinetic energy by prescribing regional variation of the coefficients, although sensitivity tests showed that the model results were affected by arbitrary (homogeneous) variations in their magnitude[20]. As meteorologists had learned with the much broader atmospheric weather systems, the problem could be largely eliminated by using a computer powerful enough to resolve the eddies. The arrival of a new generation of supercomputers in

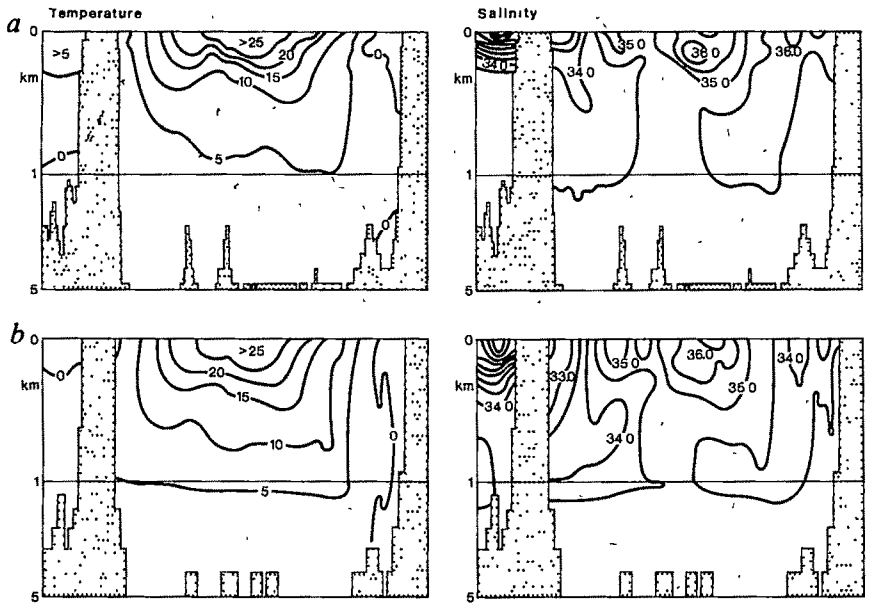


Fig. 5 The distribution of temperature and salinity in a vertical meridional section through the Pacific Ocean: *a*, from the Princeton model; and *b*, from hydrographic data[20]. The similarities in *a* and *b* are encouraging, but the difference in the depth of the 10 °C isotherm indicates that the model does not describe upper ocean ventilation adequately for decadal climate prediction.

the mid-1970s made it possible to test that hypothesis for a small ocean basin. Holland[25] demonstrated that the eddies influence the mean circulation and showed that the mean circulation is affected by bottom topography and the density distribution through the action of the eddies. Several modellers are exploring more sophisticated parameterizations designed to permit progress without resolving the eddies (J. S. A. Green, personal communication), but it is generally believed that they will have to be resolved in future climate models. The present generations of computers makes that impossible except in limited regions, or idealized ocean basins. But in designing WOCE we are looking forward to models that will be in operation at the end of the century. By then there will be two more generations of computers, with at least a factor of 100 in power, making global eddy-resolving models a practical proposition.

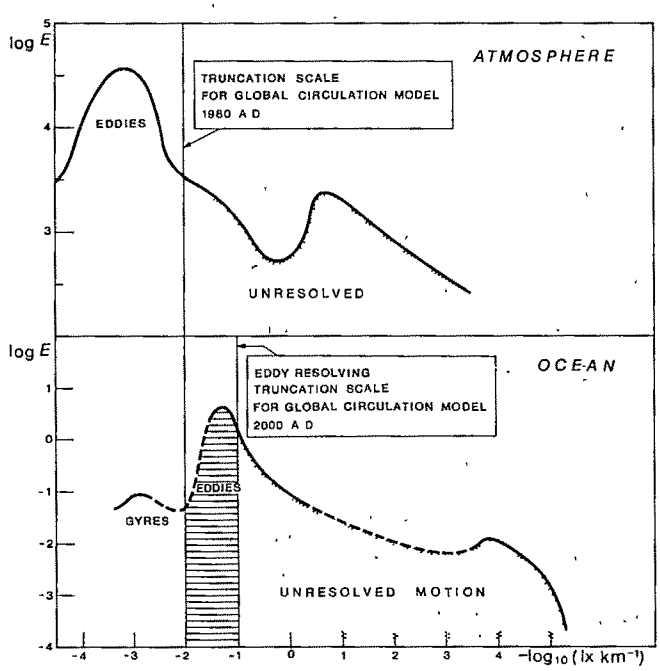


Fig. 6 Typical spectra of motion in the ocean and atmosphere showing the energy peaks associated with eddies. The eddy energy must be resolved in models of the global circulation. Existing computers permit a truncation scale of 100 km, which resolves eddies in the atmosphere, but not in the ocean. The continuing increase in computer power will permit progressively smaller truncation scales until, by the end of the century, it should become possible to resolve the ocean eddies.

Another source of error is the parameterization of vertical mixing in the statically stable interior of the ocean. In the world ocean model, this is based on a vertical eddy diffusivity for scalars and a vertical eddy viscosity for momentum. Research into small-scale transport processes has cast doubt on the efficacy of such parameterization. While many details remain to be settled, there are several processes with different transport characteristics: scalar mixing is effected by billow turbulence[26], double diffusive convection[27] and cabelling[28]; momentum transport is effected by internal waves[29] and quasi-geostrophic eddies[30]. Analysis of scalar distributions measured during large-scale geochemical surveys have emphasized the need to distinguish between vertical and diapycnic mixing[31]. Numerical techniques have been proposed to avoid this source of error by coordinate transformation[32] or, more drastically, by reformulating the circulation models in terms of isopycnic rather than isobaric surfaces[33]. Such techniques will not only improve the prediction of scalar diffusion by unresolved motions, but they also simplify the treatment of potential vorticity conservation, which is the theoretical linchpin of eddy and gyre scale dynamics[34].

Climate models are particularly sensitive to the parameterization of boundary layer processes, which control the deep ventilation of anomalies entering the ocean at the surface, and, conversely, the mixed layer temperature and therefore the fluxes of energy, water and gases into the atmosphere[36]. The standard practice in ocean circulation models is to treat the boundary layer physics one-dimensionally[37]. That is useful for simulating the response of sea-surface temperature and salinity to the seasonal variation of surface energy and water fluxes, but it does not take account of advection of heat and water by ocean currents, the essence of global climate[17]. Nor is it possible with such boundary layer parameterization to predict the depth of the mixed layer in winter, when surface water mixes deepest (Fig. 7). As a result, it is not possible to calculate the regional and temporal variation of annual water mass transformation in the boundary layer, a crucial mechanism for decadal climate change. Recent work by the Princeton group has revealed the sensitivity of deep-water ventilation to the depth of winter mixings[23,24]. It was shown that simulation of the observed distribution of tritium (from nuclear bomb tests) in the North Atlantic was greatly improved by artificially making the mixed layer in winter have the regional distribution deduced from observations. The need for accurate parameterization of boundary layer processes will become increasingly important when climate models are based on freely coupled ocean–atmosphere circulations, for which the present expedient of prescribing the seasonal variation of mixed layer temperature, salinity and depth will no longer be appropriate.

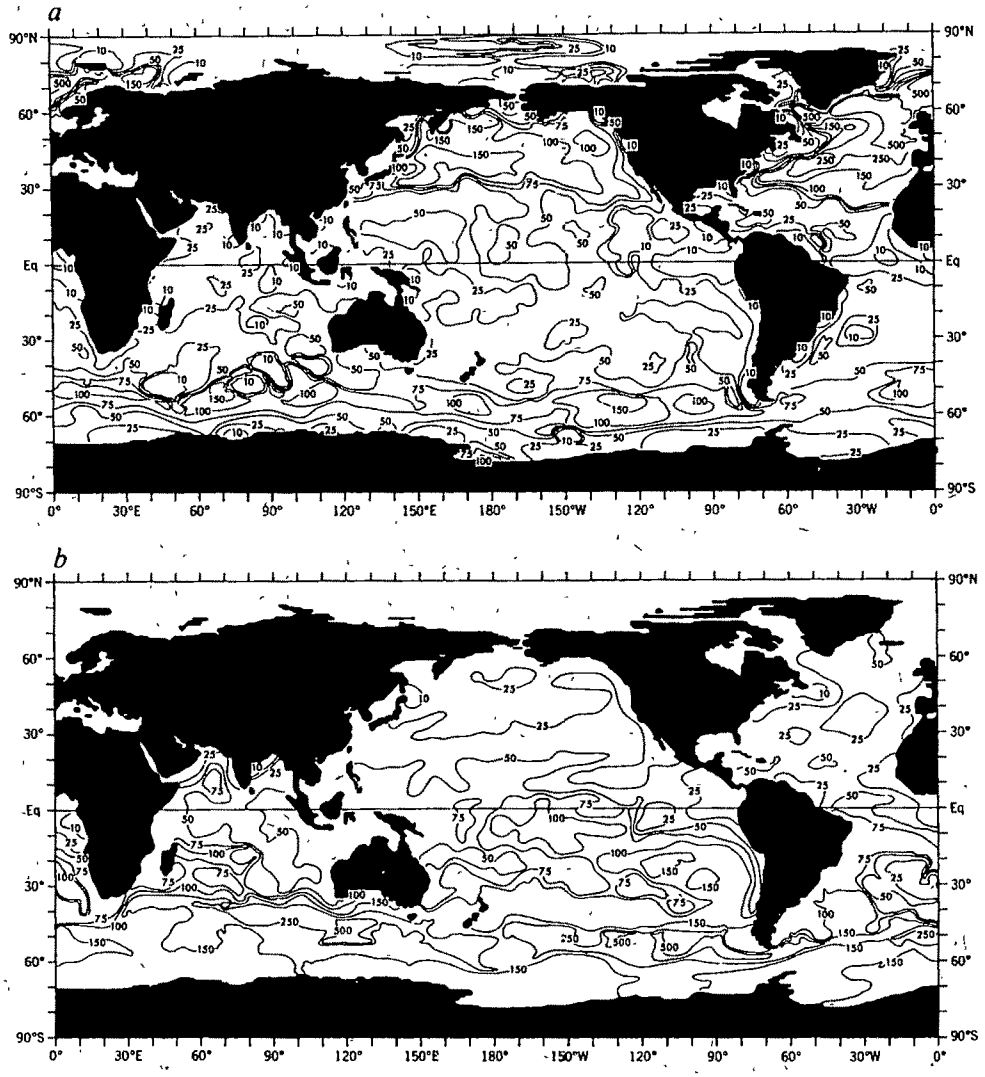


Fig. 7 The climatological mean distribution of the depth of the oceanic mixed layer at the end of winter, as deduced from density profiles[44], has been used as a parameter in models designed to simulate the penetration of anomalies from the atmosphere into the interior of the ocean[23,24]. *a*, March; *b*, September.

Future models

Increasing computer power will improve the resolution of ocean circulation models and include more elaborate parameterizations of unresolved processes. Faced with such opportunities, the modeller has to ask how much emphasis to give to processes competing for each increment in computer power. His decisions are influenced by three factors: (1) theoretical ideas illustrated by process models; (2) the availability of data to run the model with new features; and (3) the availability of data to test the resulting model predictions. It is also important that the modeller has a specific goal in mind.

The ultimate goal for modelling in WOCE is decadal climate prediction, which emphasizes those aspects of ocean circulation that influence oceanic feedback to the atmosphere over timescales of 10 or 100 years, and stresses the coupling of the global circulations of the ocean and atmosphere. The theoretical ideas that will most strongly influence WOCE model development will be those concerned with the oceanic boundary layer, the ventilation and circulation of the thermocline (the warm water sphere), and the fluxes between basins which give the ocean its global integrity. The problem facing the modeller is to assimilate the many advances made in recent decades in our understanding of processes that influence these aspects of the large-scale circulation, and to decide how much weight to give to each as the model evolves. A powerful theoretical tool for making such decisions is provided by Ertel's concept of potential vorticity conservation[34]. The full implications of the concept are still being investigated, but it has already been shown that most aspects of the dynamical state of the ocean can be determined if one knows the distribution of potential vorticity along isopycnic surfaces and the density distribution along the boundary[35]. By focusing on the distribution of potential vorticity along isopycnic surfaces, it becomes possible to assess the relative importance (for large-scale circulation) of a multiplicity of transient motions, including the quasi-geostrophic eddies and fronts, convection in the boundary layer (due to surface cooling) and in the interior (due to double diffusion), turbulence in the mixed layer and the interior, and so on. It seems likely that potential vorticity will be the dynamical touchstone of WOCE, just as geostrophy was for the great oceanographic expeditions of previous generations.

The data needed to run ocean circulation models include: (1) the topography of the sea floor as lower boundary condition; (2) the distributions of velocity, temperature, salinity and selected chemicals as initial conditions; and (3) the global patterns of seasonally varying fluxes of momentum, energy, moisture and gases at the ocean surface interface as upper boundary conditions. The bathymetry is already known to the required resolution of 30 km in some regions[38,39], but remains undersampled in most of the ocean. It is significant that 70 unknown seamounts were discovered by analysis of the Seasat altimeter data[40,41]; geophysicists predict many more. The accurate mapping of passes through which deep water passes between ocean basins presents a continuing problem.

In a recent census of the world ocean waters, Worthington[42] assessed the accumulated hydrographic data base available in the World Data Centres. There are large gaps in the Southern Hemisphere that have to be spanned by interpolation. The uncertainty in the mean heat content and total mass of salt is important even when the model is integrated from a

'homogeneous, motionless' initial state. The alternative of initializing the model, as meteorologists do in weather forecasting, with the best available description of the present day distributions is more challenging[43]. That requires a knowledge of the global distributions. The first systematic world analysis of temperature, salinity and oxygen distributions (with a resolution of $1°\times1°\times33$ layers) has recently been published[44]. The potential of surface pressure maps from altimetry for initialization of ocean circulation models is being investigated[45]. Looking further ahead, acoustic tomography[46] may provide the synoptic data required for model initialization.

The upper boundary condition for ocean circulation models used in decadal climate prediction will come from the atmospheric general circulation models (GCMs) coupled to them. At present, there are design faults in atmospheric GCMs which lead to large errors in the surface fluxes they predict[11]. Attention is now being given to identifying and eliminating the causes of those errors. Meanwhile, the ocean modeller must proceed in the uncoupled mode, using observed surface fluxes as the upper boundary condition. At present, they are calculated from ship meterological observations using formulae which have been calibrated against measurements made during air-sea interaction experiments such as JASIN 1979 (ref. 47). The unacceptably large uncertainties in the surface fluxes so determined have been widely discussed[3]. They represent a major source of error in predictions of ocean circulation models. There is a very real risk of rubbish in: rubbish out. Nevertheless, the global analysis of seasonally varying surface fluxes derived from ship observations represents the state of the art. Precipitation is notoriously difficult to estimate at sea and published distributions have to be treated with caution[48,49]. Some modellers, faced with the uncertainty in maps of surface fluxes of energy and water, have used the better observed distributions of surface temperature and salinity. That has permitted useful progress with many aspects of circulation modelling, but it is one stage further removed from the goal of coupled atmosphere–ocean climate models. The early attempts at developing coupled models have been based on a simpler parameterization of the surface fluxes based on the newtonian cooling law[19].

Summarizing, the existing descriptions of bathymetry, hydrographic distributions and surface fluxes needed to run ocean circulation models already pose difficulties and it is becoming increasingly urgent to improve them so as to avoid the risk that ocean circulation models with greatly improved resolution and physics fail to realize their potential because of inadequacies in the data used to run them. Improvements are needed in accuracy, spatial and temporal resolution and in global coverage.

Testing the models

There are two strategies for using observations to test models. The first focuses on products closely related to what the customer wants, in our case decadel climate forecasts. The second focuses on internal properties of the system being modelled; does the circulation look right? The weather forecaster can follow the first approach. The absence of adequate time series from the past, and the need to make predictions before another century has passed, forces the oceanographer to concentrate on the latter. In designing WOCE, we must choose to observe those aspects of the ocean circulation that offer the best prospect of testing the plausibility of models that will be working at the end of this century.

The requirement is for global models that treat the circulation around the world ocean in its totality. But the present state of oceanographic knowledge is uneven, and even the major advance expected as the result of WOCE cannot realistically be expected to raise our level of knowledge of all regions to the highest level. Special emphasis will be placed during WOCE on filling the most glaring gaps in our data base, but the resources available are limited and will be deployed in a way that offers the best prospect of testing future models, not simply on a regular grid. The pragmatic criterion for deciding what constitutes useful testing of a new model depends on pushing back the frontiers of our state of knoweldge. The detailed specification varies considerably from region to region. In some places, such as the North Atlantic, our knowledge of the circulation is relatively advanced and we can proceed to specify errors in measurements of, for example, the heat flux. Elsewhere, we are still relatively ignorant of the general character of the circulation; for example, we have neither documentation nor theory concerning the variability of the Antarctic circumpolar current, and we simply do not know how it connects with the circulations in the Pacific, Atlantic and Indian Oceans to the north. In such cases, WOCE must be designed to reveal the unknown; comparison with model predictions will have to be more qualitative than quantitative. The major discoveries of WOCE are expected to come from the Southern Hemisphere, which has been poorly sampled in the past, for obvious logistic reasons.

Broadly, the procedure will be to extract products from the WOCE data set that can be compared with equivalent products generated by the prognostic models. At the simplest level, it is useful to compare the distributions of temperature, salinity and dissolved chemicals (see Fig. 5). The same goes for the patterns of mean and variance of the surface pressure distribution measured by satellite altimetry. The velocity field presents greater difficulties because it must be calculated from a diagnostic model supplied with scalar distributions. Classical attempts at diagnostic modelling (reviewed in ref. 50) involved arbitrary assumptions. Recently, there have been significant advances in the application of inverse theory to the design of diagnostic models that extract products from oceanographic data, with explicit statements about the errors in the data and the tolerance to which dynamical constraints apply[51]. Once the velocity field has been diagnosed, it is possible, in principle, to compute the fluxes of heat, water and chemicals, and to establish the relative contributions of the different classes of motion to those fluxes (that is, the Ekman transport in the mixed layer, the wind-driven gyre circulations, the meridional circulation driven by deep convection at high latitude, and the eddies). By comparing the observed distributions of dissolved chemicals with those computed using a model that incorporates the WOCE empirical velocity field, it will be possible to assess the stationarity of the circulation on the ventilation timescales of the different regions, and so resolve the present uncertainty as to the origin of water in the deep Pacific; is it a fossil of an earlier climate or in equilibrium with our present climate? Inverse theory warns us that it will not be easy to determine velocities and fluxes accurately. In general, even with the great increase in data we expect from WOCE, the system will be under-determined, and model testing will be more effective if we concentrate on collecting data that will permit us to calculate a few products accurately. Compared with the richness of detail meterologists have following their global experiment, the number of such products expected from WOCE may seem discouragingly small, but they will provide powerful constraints on the models at the global level.

On a smaller scale, it is necessary to test the parameterizations of motions that cannot be resolved even in the models running on twenty-first century computers. Here the comparison will be between observed large-scale indicators of those motions, for example, the annual maximum depth of the mixed layer, which is crucial in parameterization of the ventilation of properties acquired in the boundary layer into the interior of the ocean[17]. Comparison of isopycnic potential vorticity distributions will provide one of the crucial tests of the model parameterizations of the effects of internal mixing on large-scale circulation. It is emphasized, however, that WOCE will not incorporate any detailed process studies related to particular classes of mixing: the experiment will deploy all available resources on the largest scales of motion.

Summarizing, the existing database tests models by revealing errors in scalar distributions, but provides very little information about the velocity field and the large-scale fluxes. New data are needed to extend the existing tests globally and to make it possible to test the velocity field, fluxes and internal dynamics of the models.

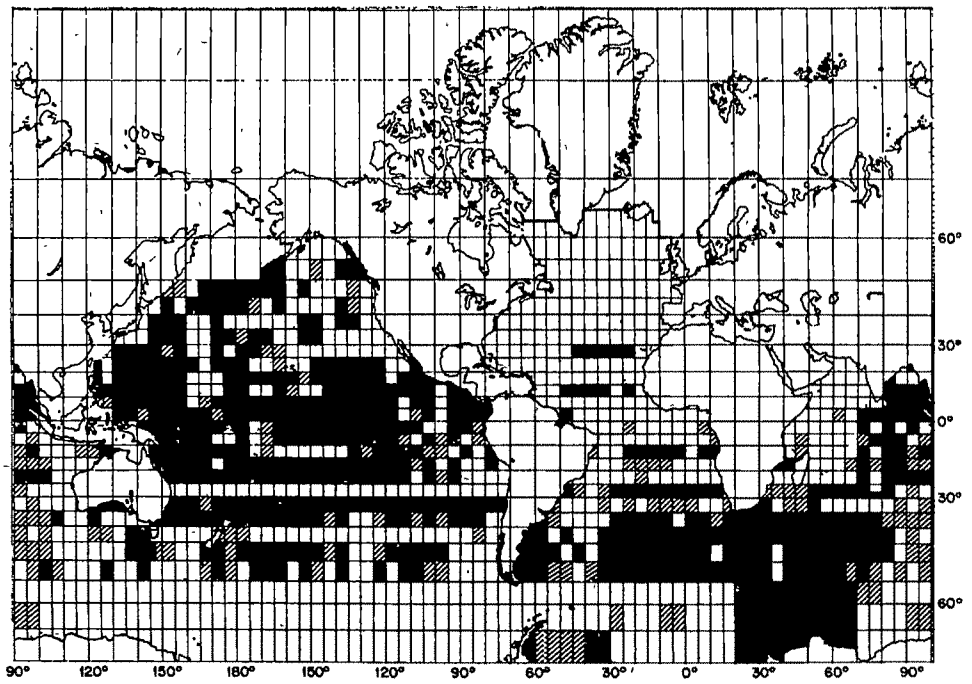


Fig. 8 The global distribution of data available for running ocean circulation models is biased towards the Northern Hemisphere, shipping lanes, fisheries zones, and against the winter months when extra-tropical air-sea interaction is strongest. The distribution of high-quality hydrographic data[42] is shown as an example. Black areas contain no suitable data; hatched areas contain data only in shallow locations.

Aims of WOCE

The discussion of model testing has revealed serious deficiencies in the existing database, which can be grouped into three categories.

First, inadequate coverage of the world ocean. The Southern Hemisphere data coverage is sparse at all seasons; everywhere there is poor coverage in winter compared with summer (Fig. 8). Merchant ship observations, which are crucial for calculating surface fluxes needed to run the models, are concentrated on the shipping lanes and biased against rough weather, when much of the air-sea interaction may be occurring. There is an urgent need for a homogeneous global data base covering all seasons.

Second, although the large oceanographic data archives available at the World Data Centres still contain untapped information, the individual measurements were often collected with other objectives in mind (such as to solve regional circulation problems) and are not well suited to testing global circulation models designed for climate prediction. There are many hydrocast profiles, but few eddy-revolving, top-bottom, trans-ocean sections.

Third, the accuracy of many measurements does not meet the specification generated by analysis of climate prediction requirements, because measurements were made originally for other purposes, for example the surface fluxes derived from ship meteorological observations. More frustrating, because it arises from sloppy practice, is the poor salinity accuracy in deep profiles from some laboratories.

The aim of the WOCE is to remedy those deficiencies by new measurements, designed specifically to meet the needs of global circulation models for climate prediction. It will take advantage of the remarkable advances that have been made in theoretical understanding of ocean circulation, in instrumentation and in design concepts since the last great international expeditions[52]. Above all, the aim will be to collect a data set that will permit a global perspective in studies of ocean circulation.

Timing of WOCE

Those are very ambitious aims and the question is, is it timely to embark on such a major project only five years hence? The answer is that four significant advances in the past decade have made it possible to take this logical next step in the physical exploration of the oceans: (1) exploration of the transient, quasi-geostrophic eddies, (2) the first computer model of the world ocean circulation, (3) new techniques for exploring the ocean from space and *in situ*, (4) recognition that improved knowledge of ocean circulation was needed before the climate impact of CO_2 pollution could be predicted. These four developments will now be discussed briefly in the context of the WOCE.

Eddies. The importance of the transient quasi-geostrophic eddies for ocean circulation had not been recognized when the last international expeditions were being planned, even though, with hindsight, there is evidence of the eddies in earlier data. Following the POLYGON, MODE and POLYMODE experiments in the past decade and associated theoretical studies, it is possible to assess the role of the transient eddies in the larger-scale permanent gyres[53]. It is also possible to design an observing strategy and data analysis techniques that take account of the eddies, whether as a dynamically important signal in their own right or as a noise on the gyres. The form of the spectrum of oceanic motion is known to a wavelength of 10 km, which is probably sufficient for designing WOCE. The recognition that general circulation models will have to resolve the eddies is a major factor in planning the experiment.

Models. The successful completion of the first computer model of the world ocean circulation, with the three-dimensional distribution of temperature and salinity, was an important milestone in oceanography. It provides the springboard for successive improvements that will eventually lead to climate prediction models. Future improvements, including resolution of the eddies, diapycnic rather than vertical mixing, and coupling of circulation and boundary layer physics, were discussed earlier. The implementation of those improvements in global models will depend on the availability of adequate computer power. The spatial resolution problem poses the greatest difficulty; a 100-fold increase in computer power is needed to achieve resolution equivalent to that currently used in weather forecasting models. Happily, there is every prospect of that being achieved by the end of this century. The most powerful machines in the world today, the Cray-XMP and Cyber 205, can perform several hundred million instructions per second; laboratories leading research into eddy-resolving ocean circulation models are already planning to order computers that can perform more than a thousand million instructions per second. Even if some of the more bullish predictions of the rate of increase in computer power prove optimistic, there is no doubt that early in the next century, ocean circulation modellers will need a data set to test global eddy-resolving models. The WOCE cannot be completed before 1995 at the earliest and, judging by the time it has taken to complete the GWE data set, the WOCE data set will barely be ready for use by the end of the century—none too soon for testing models using fifth-generation computers.

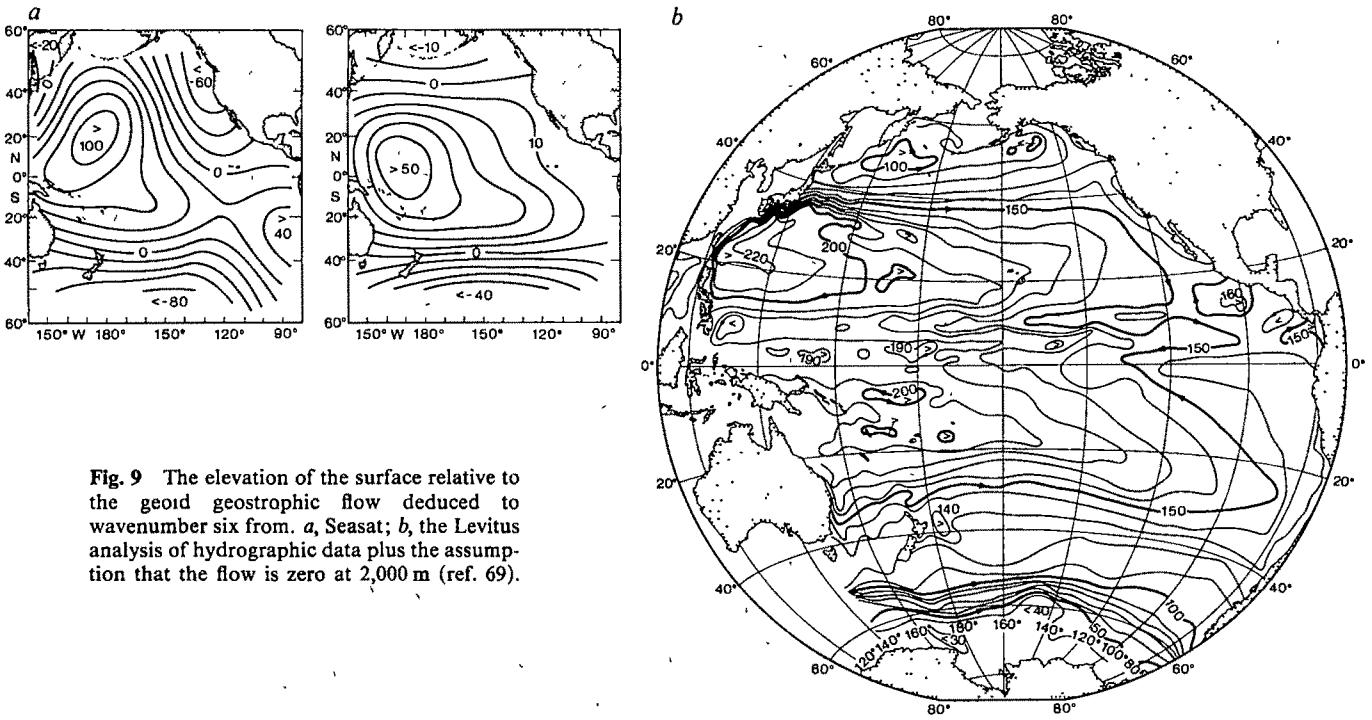


Fig. 9 The elevation of the surface relative to the geoid geostrophic flow deduced to wavenumber six from. *a*, Seasat; *b*, the Levitus analysis of hydrographic data plus the assumption that the flow is zero at 2,000 m (ref. 69).

New tools. The Atlantic expedition during the IGY was based almost entirely on instruments lowered from research ships. The most revolutionary tool was the bathythermograph, which could give a shallow temperature profile without stopping the ship. The IGY data set is the mainstay of contemporary calculations of Atlantic circulation. Research ships will continue to have the central role in ocean exploration for the foreseeable future, and they will provide the core of the WOCE data set. New techniques will improve the efficiency of ship use. The increasing use of expendable instruments (bathythermograph, current profiler) has made it possible to supplement the limited research fleet with ships of opportunity. Systematic surveying of temperature anomalies has been undertaken in this way during North Pacific Experiment[54], and is now being used in the Tropical Ocean and Global Atmosphere[55] project.

During the 1970s there was also a rapid development of moorings and drifters for circulation studies. Moorings can now be deployed for up to a year, making it possible to explore variability with periods much longer than the eddy lifetimes[56]. Surface drifters tracked by satellite[57] and deep drifters tracked acoustically[58] can be followed for many months. Pressure gauges deployed on the ocean floor for several months have been used to map the tides[59]. The accumulated number of buoy-months has already made it possible to map velocity mean and variance in the North Atlantic[60]. Theoretical studies have helped to clarify the interpretation of the quasi-langrangian tracks of these drifters[61].

Methods of chemical analysis of water samples collected by research ships have advanced rapidly in the past decade. It is possible to measure the concentrations of chemicals present at very low concentrations with far smaller samples than hitherto. And many of the chemicals can now be analysed at sea. Mass spectroscopy has revolutionized the measurement of tritium and other elements previously detected by counting radioactive decay[62,63]. Those advances have made it possible to undertake extensive surveys of chemical distributions in the world ocean: first GEOSECS (Geochemical Ocean Sections Study)[64] and more recently TTO (transient tracers in the ocean)[65]. These chemicals supplement the variables (temperature, salinity, oxygen and nutrients) used traditionally to infer the circulation[52,66].

The most spectacular advance in ocean observation has come from the demonstration by Seasat during a 100-day mission in 1979 that satellite radar and passive microwave techniques now offer the accuracy needed for ocean circulation research[67,68]. The success of the radar altimeter in measuring the shape of the ocean to a precision better than 10 cm opens the door to global mapping of the surface geostrophic velocity (Fig. 9), thereby allowing oceanographers to dispense with the classical and unwarranted assumption that the flow goes to zero at some arbitrary deep level[69]. The advent of satellite altimetry and plans for future altimetric missions provided the initial impetus for WOCE. The technique offered the prospect of a truly global perspective of the ocean circulation for the first time. The timing of the experiment has from the start been based on the launch date of the next altimetric mission. At present, it seems likely that the European Earth Resources Satellite ERS-1 and the American-French Topex-Poseidon will be launched in 1990 and operated for 3–5 years. The success of other satellite techniques has also influenced the early planning of WOCE. In particular, the radar scatterometer, which promises to provide systematic global measurements of the wind stress on the ocean for the first time[70]. The measurement of sea-surface temperature by infrared radiometer has had a chequered past, but at last seems to be yielding measurements accurate to 0.6 K, with the prospect of improvement to about 0.3 K in the future[71]; that begins to be useful for WOCE.

Other techniques showing promise for WOCE include acoustic tomography, which has mapped eddy patterns in an early sea trial[46], and acoustic Doppler current profiling, which might be deployed widely on merchant ships to map currents in the top few hundred metres of the ocean[72]. Increasing attention is being paid to collecting data from research ships moving at speed (in contrast to the classical technique of profiling on station): undulating profilers derived from the Batfish have been used to collect upper ocean sections several megametres long[73]. Baker[74] has reviewed these and other new techniques that lead us to believe that some of the old problems preventing oceanographers from observing the ocean globally are being solved.

CO_2 pollution. Early calculations of the climate response to CO_2 pollution of the atmosphere suggested that equilibrium with twice the present concentration would be achieved when the atmosphere was on average about 2 K warmer than now, with a smaller rise in the tropics but up to 8 K at high latitudes, and an associated change in the pattern of precipitation[75]. There was speculation that the temperature rise might cause the West Antarctic ice sheet to melt, raising sea level by several metres[76], and that the change in rainfall pattern might have a major impact

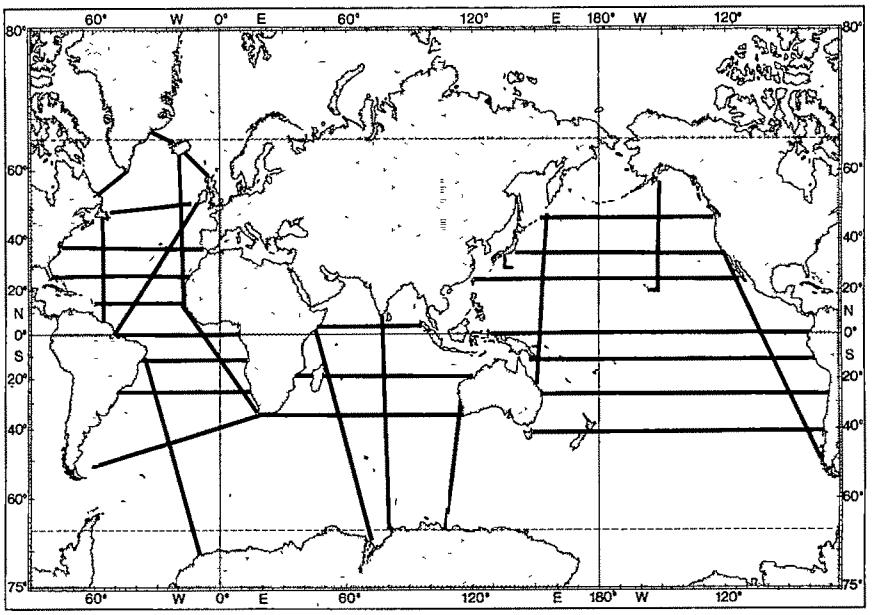


Fig. 10 One possible distribution of trans-ocean hydrographic sections for WOCE based on a realistic estimate of ship time likely to be forthcoming from existing resources available to the world oceanographic community (ref. 78). Additional ship resources will be needed to undertake the WOCE hydrographic and tracer programme in the Southern Hemisphere, which is not adequately covered by the sections identified in this map.

on agriculture in some countries[77]. These predictions were sufficiently alarming to create a demand for research into predicting decadal changes of climate (CO_2 concentration being predicted to double within 50 years). Further modelling made it clear that the rate and global pattern of atmospheric temperature rise will be largely determined by sea-surface temperature response to the changing infrared flux from the atmosphere. It was realized that early models based on heating limited to the mixed layer, which gave a response time of a few years, were wrong. It is necessary to take account of the subduction of water from the mixed layer into the interior by Ekman pumping and deep convection in winter[17]. That spreads the heat input over a much greater water depth, delaying the attainment of an equilibrium sea-surface temperature rise for decades, and allowing the ocean circulation to distribute the thermal anomaly widely, perhaps even between ocean basins. Unfortunately, the present state of ocean circulation modelling, and especially the treatment of the subduction process, is inadequate to the task of predicting accurately the transient thermal response of the ocean to the changing surface infrared flux due to CO_2 pollution. The early timing of WOCE is, in part, a response to the sense of urgency generated by those concerned with predicting climate response to CO_2 pollution.

Experimental strategy

The primary goal of the experiment is to develop ocean models useful for predicting decadal climate change and to collect the data necessary for testing them. The specific objectives are to determine and understand on a global basis the following aspects of the world ocean circulation and their relation to climate: (1) The large-scale fluxes of heat and fresh water and their divergences within 5 years, and in addition their annual and interannual varaiblity. (2) The inputs and fluxes of potential vorticity. (3) Components of oceanic variability on months to years, megametres to global scale, and the statistics on smaller scales. (4) The rates and nature of formation, ventilation and circulation of water masses that influence the climate system on time scales ranging from 10 to 100 years.

The experimental design strategy being followed for WOCE offers three perspectives on the circulation: the global scale; the links between the separate ocean basins; and inside the basins. Inevitably, there will be overlap between those three perspectives, but the detailed experimental design, and the precise specification for similar measurements, will be different.

The field programme will be intensive, with a limited duration related to the planned mission lives of the next generation of ocean observing satellites, which provide the first opportunity to collect global observations. The expected launch year of those satellites is 1990, and their planned lifetimes are 3–5 years. The intensive oceanographic programme of WOCE will, therefore, concentrate on the period 1990–95, with the possiblity of adjustment according to changes in satellite launch dates. Those measurements in the ocean that need to be made simultaneously with the satellite measurements must be concentrated into that period. Other measurements (for example, some aspects of the deep tracer surveys) can safely start earlier and continue after the 1990–95 period.

The fact that the intensive field programme will extend over several years does not mean that the resulting data set will constitute an average or synoptic description of the world ocean during that period. The satellite data will come closest to the ideal of a time series of synoptic observations. Most of the *in situ* observations will be made once, sequentially as part of a rolling programme extending over 5 or more years. A few measurements will be repeated to check for significant changes, and there will be a small number of time series. There is no single name that describes such a non-synoptic sample, which will inevitably alias most of the changes occurring within the intensive field phase. The word 'snapshot' captures something of the spirit in which the WOCE data set will have to be analysed, provided one bears in mind the photographic problems of camera shake, focal plane distortion, and so on. Despite these problems of interpretation, the WOCE snapshot will be far more coherent and closer to being synoptic than the existing data archive accumulated over several decades. It will, therefore, represent a significant advance in sampling technique, as well as in global coverage. A conscious effort will be made in designing the experiment to minimize the remaining problems of aliasing. Selected measurements will also be continued for a longer period to discover how representative was the WOCE snapshot of the ocean, given its broad spectrum of natural variability.

WOCE design

The development of an experimental design soon becomes a juggling act between the desirable and the possible. This section outlines the principal ingredients of the experiments, as proposed by Wunsch[78]:

(1) The fields of surface forcing as determined by (i) satellite scatterometers (anticipated spacecraft are the European Space Agency's ERS-1 and the US National Remote Ocean Sensing Satellite NROSS) for wind stress; (ii) conventional meteorological analyses for wind stress and heat and moisture fluxes based on improved atmospheric modelling efforts and an upgraded surface observational system using drifters in otherwise uncovered regions.

(2) The field of oceanic surface topography (the oceanic surface pressure boundary condition) determined by (i) satellite altimeters (anticipated spacecraft are ERS-1 and the US NASA Topex Mission); (ii) tide gauge network on coasts and oceanic islands.

(3) Global hydrography from ships using both highest quality conductivity–temperature–pressure profilers plus developing free-fall instruments from 'research ships of opportunity' to provide both a climatological temperature–salinity data base plus continent-to-continent, top-to-bottom sections for use with dynamic method and ancillary observations and models (Fig. 10).

(4) Global measurements from ships (probably combined with hydrographic measurements) of chemical tracers, including tritium, fluorocarbons, oxygen and nutrients. These observations, along with the hydrography, provide the primary observable parameters representing the integrated (that is, time-average) behaviour of the ocean circulation.

(5) Global, but sparse, sampling of selected chemical tracers such as ^{14}C as part of the determination of time-integrated oceanic behaviour.

(6) Global, but sparse, determination of the dominant source-sink terms of the biogeochemical tracers involving transfers to and from the sea floor and continental margins by sediment traps and so on, for purposes of using the tracers.

(7) Direct observations of the flow fields over large areas for long time periods by 'clouds' of neutrally buoyant floats and surface drifters, both communicating directly with satellites.

(8) Measurements of large areal averages for long times, of heat content, velocity and vorticity and potential vorticity through moored acoustic tomography arrays.

(9) Ship of opportunity programmes involving near-surface velocity measurements through acoustic backscatter and direct near-surface density field measurements through expendable bathythermographs.

(10) A few special, regional foci involving ships, conventional moored current meters, floats, temperature recorders, tide gauges and other *in situ* instrumentation.

It is expected that it will take about 5 years to complete this measurement programme. A related programme will monitor critical aspects of the ocean over a longer period to relate that 'snapshot' of the circulation to the broad band spectrum of ocean variability.

Potential for new techniques

The WOCE design outlined above depends heavily on new techniques. How safe is it to base an experiment on new technology, and what is the criterion for rejecting promising but as yet unproven techniques? Experience gained during the GWE is relevant. The core measurements for GWE were the World Weather Watch routine synoptic observations; the equivalent for the WOCE are the research ship hydrographic and chemical surveys. Satellites were crucial for GWE, as they will be for WOCE; if present plans for altimeter and scatterometer satellites were cancelled, the WOCE would have to be postponed. Special observing systems planned for the GWE included neutrally buoyant balloons and buoys drifting on the ocean; the former failed, but the latter were successful and transformed the ability of meteorologists to analyse weather systems in the Southern Hemisphere. Special observing planned for WOCE include acoustic tomography and neutrally drifting buoys that pop up to be interrogated by satellite; both lie at the leading edge of oceanographic technology. Planners of the GWE counted on being able to improve the quality and data returns of well-established routine measurements on land; WOCE needs to do this for the meteorological measurements made by voluntary observing ships.

Looking back on the GWE, it is clear that all the observing systems that were eventually deployed in 1979 had been identified 10 yr earlier. Gaining from that experience, WOCE planning has, therefore, proceeded on the basis that no completely new techniques announced during the 1980s would be ready in time for WOCE. Most of the techniques that have been listed as needed for WOCE are the subject of intense development efforts. Perhaps the greatest uncertainty concerns the development of a new temperature–salinity profiler that can be used from research vessels not equipped with deep winches. If successful, this new instrument will make it possible to share the enormous task of the WOCE hydrographic programme among a much larger number of research institutes.

Novel techniques will be tested during the WOCE snapshot to discover their potential for cost-effective long-term monitoring of the ocean in the next century.

Conclusion

The World Ocean Circulation Experiment is an undertaking planned to support research in decadal climate variation. It will also provide a unique global perspective of ocean circulation with applications in many other aspects of marine science.

The WOCE is a major component of the World Climate Research Programme (WCRP) sponsored jointly by intergovernmental and non-international governmental organizations. The WCRP is concerned with discovering whether it is possible to predict climate on timescales of several weeks to several decades, and to determine the effect of man's influence on climate. The programme is divided into three streams, concerned respectively with climate prediction in periods of (1) months, (2) years and (3) decades. The critical scientific problem limiting our ability to predict decadal climate change is the inadequate description of and ability to model the circulation of the world ocean. The organizers of the WCRP have therefore proposed the WOCE as the principal activity within stream (3) of the WCRP. They have established the WOCE Scientific Steering Group (SSG) to plan and organize the experiment. The tasks of the SSG are to identify the primary goals and objectives of WOCE, to design a core research programme including modelling and fieldwork, to identify the resources needed to undertake the programme, and to work with international and national bodies, with research institutes and universities and with individual scientists to achieve that core research programme and to encourage the development of associated projects that support the goals of WOCE. J.D.W. is co-chairman of the SSG.

1 GARP Joint Planning Staff *The Global Weather Experiment* (World Meteorological Organization, Geneva, 1981)
2 Schlee, S *The Edge of an Unfamiliar World. a History of Oceanography* (Dutton, New York, 1973)
3 Woods, J D in *The Global Climate* (ed Houghton, J) 141–187 (Cambridge University Press, 1984)
4 Oort, A H & Vonder Haar, T H *J Phys Oceanogr* 6, 781–800 (1976)
5 Burridge, D M in *The Cage Report* (WCP-22, WNO, Geneva, 1982)
6 Campbell, G C thesis, Colorado State Univ, Fort Collins (1981)
7 Swinbank, R. *Met 0 20 Technical Note II/213* (Meteorological Office, Bracknell 1983)
8 Stommel, H *Proc. natn. Acad. Sci. U S A.* 77, 2377–2380 (1980)
9 Bryden, M L in *Time Series of Ocean Measurements* (ed Elliett, D) (WCP Rep No 21, World Meteorological Organization, Geneva, 1982)
10 Bunker, A F., Charnock, H & Goldsmith, R A *J mar. Res* 40 (*Suppl.*), 73–84 (1984)
11 Dobson, F E *et al. WCP Rep Ser No 2* (World Meterological Organization, Geneva, 1981)
12 Namias, J & Cayan, D R *Science* 214, 869–976 (1981)
13 Nuler, P P in *WCRP Pub Ser* 1, 473–485 (1982)
14 Dalmas, R J., Ascencio, J M & Legrand, M *Nature* 284, 155–157 (1980)
15 Ramathan, V *J atmos. Sci.* 38, 918–930 (1981)
16 Bryan, K., Komro, F G., Manabe, S & Spelman, M J *Science* 215, 56–58 (1982)
17 Woods, J D in *Coupled Atmosphere–Ocean Models* (ed Nihoul, J C J) Ch 34 (Elsevier, Amsterdam, 1985)
18 Bretherton, F P *Prog Oceanogr* 11, 93–129 (1982)
19 Nihoul, J C J (ed) *Coupled Atmosphere–Ocean Models* (Elsevier, Amsterdam, 1985).
20 Bryan, K & Lewis, L J *J geophys Res* 34, 2503–2517 (1979)
21 Bryan, K. *A. Rev. Earth planet Sci.* 10, 15–38 (1982)
22 Stommel, H & Fieux, M *Oceanogr Atlases* (Falmouth, Massachusetts, 1980)
23 Cox, M D & Bryan, K *J phys Oceanogr* 14, 674–687 (1984)
24 Sarmiento, G *J phys Oceanogr* 13, 1924–1939 (1983)
25 Holland, W R., Harrison, D E & Semtner, A J in *Eddies in Marine Science* (ed Robinson, A R) 379–403 (Springer, Berlin 1983)
26 Woods, J D *J Fluid Mech* 32, 791–800 (1968)
27. Schmitt, R *J phys. Oceanogr* 11, 1015–1026 (1981)
28 Turner, J S in *Evolution of Physical Oceanography* (eds Warren, B & C Wunsch) 236–263 (MIT Press, Cambridge, 1981)
29 Olbers, D, *Rev Geophys Space Phys.* 21, 1567–1606 (1983).
30 Rhines, P & Holland, W R *Dyn Atmos Oceans* 3, 289–326 (1979)
31 Rooth, C & Östlund, G *Deep-Sea Res* 19, 481–492 (1972)
32 Reid, M H *J phys Oceanogr* 12, 1154–1158 (1982)
33 Bleck, R & Boudra, D B *J phys Oceanogr* 11, 755–770 (1981)
34 Holland, W R., Keffer, T & Rhines, P B *Nature* 308, 698–705 (1984)
35 Hoskins, B., McIntyre, M & Robertson *Q Jl R. met Soc.* (submitted)
36 Woods, J D *WCRP Publ Ser* 1, 147–179 (1983)
37 Kraus, E B (ed) *Modelling and Prediction of the Upper Layers of the Ocean* (Pergamon, Oxford, 1977)

38 Hydrographer of the Navy *Bathymetry of the Northeast Atlantic* (Taunton, 1975-83)
39 *General Bathymetric Chart of the Oceans* 5th edn (UNESCO, Paris, 1977-82)
40 Dixon, T H, Naraghi, M & McNutt, M K. *J geophys Res* 88, 1563-1571 (1983)
41 White, J V, Sailor, R V, Lazarewicz, A R & LeSchack, A R. *J geophys. Res* 88, 1541-1551 (1983)
42 Worthington, L V in *Evolution of Physical Oceanography* (eds Warren, B & Wunsch, C) 42-69 (MIT Press, Cambridge, 1981)
43 Robinson, A R *et al. Nature* 309, 781-783 (1984)
44 Levitus, S *Climatological Atlas of the World Ocean* (NOAA Prof Pap No 13, U S Department of Commerce, Rockville, 1982)
45 Marshall, J C *Ocean Modelling* 57, 7-11 (1984)
46 The Ocean Tomography Group *Nature* 299, 121-125 (1982)
47 Charnock, H & Pollard, R T *Phil Trans R. Soc.* A308, 221-449 (1983)
48 Muntz, Y in *Precipitation Measurements from Space* (eds Atlas, D & Thiele, O W) (NASA Goddard Laboratory, 1981).
49 Baumgartner, A & Reichel, E *The World Water Balance* (Elsevier, Amsterdam, 1975)
50 Sarkisyan, A in *The Sea* Vol 6 (eds Goldberg, E D, McCave, I N., O'Brien, J J & Steele, J H.) 363-458 (Wiley, New York, 1977)
51 Wunsch, C in *The Global Climate* (ed Houghton, J T) 189 (Cambridge University Press, 1984)
52 Wüst, G "*Meteor*" *Report VI* (*1*) (English edn by Emery, W J, 1978) (National Science Foundation, Washington, D C, 1935)
53 Robinson, A R. (ed) *Eddies in Marine Science* (Springer, Berlin, 1983)
54 Wyrtki, K McLain, D & Patzert, W *Variability of the Thermal Structure in the Central Equatorial Pacific Ocean* (Hawaii Institute of Geophysics Rep HIG-77-1, Honolulu, 1977)
55 Gill, A E (ed) *Toga* (WCP Rep, World Meteorological Organization, Geneva, 1983)
56 Elliott, D J (ed.) *Time Series of Ocean measurements* (WCP-21, World Meteorological Organization, Geneva, 1982)
57 McNally, G J *J geophys Res* 86, 8022-8030 (1981)
58 Rossby, H T, Riser, S C. & Mariano, A J in *Eddies in Marine Science* (ed Robinson, A) 66-91 (Springer, Berlin, 1983)
59 Cartwright, D E *Proc. 9th int Symp Earth Tides*, New York (Springer, Berlin, 1983)
60 Richardson, P L *J geophys. Res* 88, 4355-4367 (1983)
61 Haidvogel, D, Robinson, A R & Rooth, C in *Eddies in Marine Science* (ed Robinson, A) 481-491 (Springer, Berlin, 1983).
62 Jenkins, W J *J mar Res* (*Suppl*) 40, 265-290 (1982)
63 McCartney, M S *J. mar Res* (*Suppl*) 40, 427-464 (1982)
64 Broecker, W in *Evolution of Physical Oceanography* (eds Warren, B & Wunsch, C) 434-460 (MIT Press, Cambridge, 1581)
65. Rother, W *WCRP Publ. Ser* 1, 459-471 (WMO, Geneva, 1983)
66 Worthington, L V *On the North Atlantic Circulation* Ch 13 (Johns Hopkins University Press, Baltimore, 1976)
67 Kirwan, A D, Ahrens, T J & Born, G H *J geophys Res* 88, 1529-1952 (1983)
68 Woods, J D *Phil Trans. R. Soc.* A309, 337-359 (1983)
69 Tai, C K & Wunsch, C *Nature* 301, 408-410 (1983)
70. Guymer, T H *Phil Trans R. Soc.* A309, 399-414 (1983)
71 Harries, J E, Llewllyn-Jones, D T, Minnett, P J, Saunders, R W & Zavody, A M *Phil. Trans R. Soc.* A309, 381-395 (1983)
72. Crocker, T R *Deep-Sea Res.* 30, 449-467 (1983)
73. Leach, H, Minnett, P & Woods, J D *Deep-Sea Res.* (in the press)
74 Baker, D J in *Evolution of Physical Oceanography* (eds Warren, B & Wunsch, C) 396-433 (MIT Press, Cambridge, 1931)
75 Manabe, S, Bryan, K. & Spelman, M. J *Dyn Atmos Ocean* 3, 393-426 (1979)
76 Wilson, A T *Nature* 201, 147-149 (1964)
77 Kellogg, W in *Climate Variations and Variability Facts and Theories* (ed Berger, A) 601-621 (Reidel, Dordrecht, 1981)
78 Wunsch, C in *Proc. Workshop on Global Observation and Understanding of the General Circulation of the Oceans* (U S National Academy of Sciences, Washington D C, 1983)

ARTICLES

Cosmic γ rays and the mass of gas in the Galaxy

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We make the case that the cosmic-ray particles that produce cosmic γ-rays in the 100-MeV region can penetrate most of the dense clouds of gas that seem to contain much of the mass of gas in the Galaxy. We determine a probable form for cosmic-ray intensity as a function of Galactic position and use the measured γ-ray intensities to derive the surface density of gas as a function of position. Our estimate of $\sim 6 \times 10^8$ $M_\odot$ for the total mass of molecular hydrogen compared with 1×10^9 $M_\odot$ for atomic hydrogen in the inner Galaxy is a significant change in the contemporary perspective.

THE state of the interstellar medium (ISM) has been investigated in all wavelength regions. We describe here the distribution of gas on the galactic scale from γ-ray studies and put forward other recent supporting evidence.

Information about the magnitude of gas density and its division between the major components, atomic H I and molecular hydrogen, is relevant to several problems in astronomy, one being the manner in which the cosmic-ray intensity varies with position in the Galaxy. The gas distribution must be known to interpret the diffuse γ-ray flux. γ Rays, produced by the interaction of cosmic rays with gas in the ISM, are an ideal tracer because they are not absorbed by the gas.

The distribution of H I is known reasonably well from observations of the 21-cm line. The situation with the other major gas component, H_2, is less clear, however, because the observations are indirect, obtained by detection of ^{12}CO and ^{13}CO. It is thought that the CO is excited by impacts with H_2 molecules and various arguments can be used to relate the CO densities to those of H_2 (see, for example, the comprehensive analysis of Sanders *et al.*[1]; to be referred to as SSS).

A detailed study of the H_2 problem has been made by SSS under the usual assumption of radial symmetry (Fig. 1). This can be regarded as the 'conventional' estimate of the surface density of H_2, $\sigma_{H_2}(R)$; here, we estimate this quantity by quite different means (see Fig. 1 legend).

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The γ-ray view

In the absence of a significant contribution of γ rays from discrete sources[2-4], the relation between the cosmic-ray intensity (I_{CR}), the surface density of gas and the observed γ-ray intensity is straightforward.

First, we examine very local molecular clouds where I_{CR} is presumably the same as that at the Earth and determine the best value of α, the conversion factor relating the measured quantity from CO measurements, $\int T\,dV$, (where T is antenna temperature and V velocity) to the column density of gas, $N(H_2)$. $\alpha = N(H_2)/\int T\,dV$ in units of 10^{20} atoms $cm^{-2}\,K^{-1}\,km^{-1}\,s$ (note that some authors use 'X' $= \alpha/2$ in units of molecules $cm^{-2}\,K^{-1}\,km^{-1}\,s$). The value can then be used in the inner Galaxy, suitably modified according to our view of interstellar chemistry. Second, we use the dependence of I_{CR} on radial distance (R) in the outer Galaxy to imply its probable dependence on R in the inner Galaxy, and thereby determine the total gas surface density independently.

We start by studying the local clouds, particularly those in Orion. As well as determining a value for α, we must show conclusively that the cosmic rays are able to penetrate most of the gas in the clouds.

Local molecular clouds

Several γ-ray studies of the Orion clouds have been made already, but here we follow the analysis of Houston and Wolfendale[5] and concentrate on the two clouds A and B. The question of a possible exclusion of cosmic rays from the dense parts of

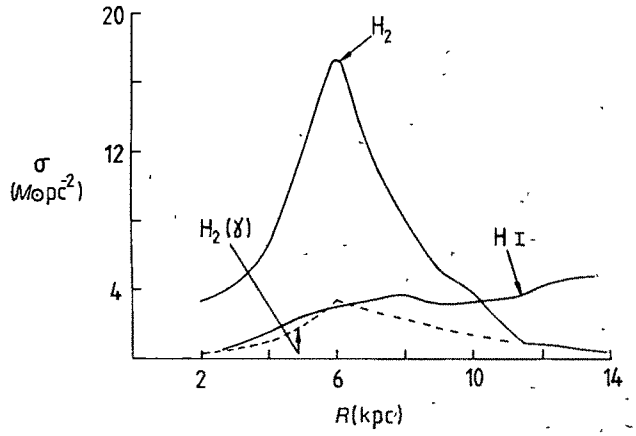


Fig. 1 Dependence of the surface density of H_2 and H I on Galactocentric radius, R. H_2 is estimated in ref. 1; in other analyses different conversion factors have been applied, resulting in maximum densities varying from about 13 $M_\odot\,pc^{-2}$ (ref. 8) to 32 $M_\odot\,pc^{-2}$ (ref. 35). The conversion factor for $^{12}CO \rightarrow H_2$ used in ref. 1 in deriving H_2 is $\alpha = 7.2\times10^{20}$ atoms $cm^{-2}\,K^{-1}\,km^{-1}\,s$. The curve for H I is the mean of derivations in refs 8 and 9; both use the basic Berkeley data[36]. $H_2(\gamma)$ is our own estimate of $\sigma_{H_2}(R)$.

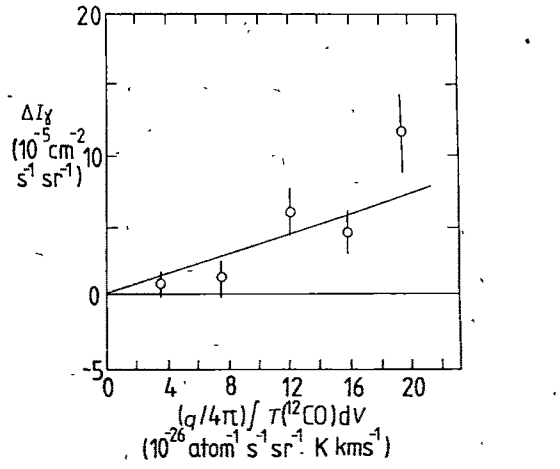


Fig. 2 Correlation of γ-ray intensity ($E_\gamma > 100$ MeV) with $\int T(^{12}CO)\,dV$ for the Orion clouds A and B (after ref. 5). ΔI_γ is the γ-ray intensity less the predicted contribution from CR interacting with H I along the line of sight. The CO data are from Kutner *et al.*[37] and the areas adopted were within the 1 K km s^{-1} contours. The γ-ray intensities are from the COS B experiment[4]; $q/4\pi$, the emissivity per H atom, has been taken as 2.0×10^{-26} atom^{-1} s^{-1} sr^{-1}, this value being the mean of various 'high latitude' estimates. The mean slope gives $\alpha = 3.7\times10^{20}$ atoms $cm^{-2}\,K^{-1}\,km^{-1}\,s$; the errors shown are statistical.

the clouds has been examined[5]. Although the cosmic-ray energies are probably sufficiently high for exclusion not to be dramatic, as suggested theoretically by Skilling and Strong[6], nevertheless an experimental demonstration is essential.

The result can be seen in Fig. 2 and its legend. Note that there is near linearity of ΔI_γ with $\int T\,dV$; if linearity is assumed, α follows as the gradient of the line and is found to be 3.7 ± 1.0 atoms $cm^{-2}\,K^{-1}\,km^{-1}\,s$. This value of α is much less than that in Fig. 1 (3.7 compared with 7.2); it is in fact a little smaller than the value we used previously (for example, 4.6 in ref. 7, following Gordon and Burton[8]), although Li *et al.*[9] made the case for $\alpha = 2.3$, locally, from various other arguments. A more detailed discussion of these α values is given later.

Figure 2 shows that there is no evidence for cosmic-ray (CR) exclusion from the densest parts of the clouds (on the angular scale of the COS B observations). If anything, there might be an excess of CR in the densest parts. If the γ-ray excess were attributed to an excess of cosmic-ray particles (or unresolved γ-ray sources), then the true value of α for the $CO \rightarrow H_2$ conversion would be significantly smaller than in Fig. 2, but the evidence is not yet strong enough for this assumption.

Other local clouds

The 'local' CR intensity adopted for Orion can probably be used out to ~1 kpc (despite evidence for local gradients[10]); the analysis of Issa and Wolfendale[11,12] examined the local clouds. The objects in this range comprise Taurus, Corona Austrina, Ophiuchi and Gum and Monoceros OBI as well as Orion. Masses have been estimated both with and without the local thermodynamic equilibrium (LTE) condition and using $\alpha = 4.6$, and suitable allowance has been made for H I and ionized gas where appropriate. The lower limits to the mass (that is, adopting LTE) now seem more appropriate and the results of Issa and Wolfendale[11,12] show that the average value of the CR enhancement factor (F is the ratio of I_{CR} in the cloud to I_{CR} locally) needed to satisfy the γ-ray data is $F \simeq 0.5$. Thus, a re-analysis of this work gives $\alpha = 2.3$, somewhat smaller than that derived in the previous section.

Here, we have presented evidence for two local values of α, 3.7 and 2.3. Of these, the first is statistically firmer, but there is almost certainly a correction to be applied because the 'metallicity' M (defined here as the ratio of O to H, normalized to the local value) is lower in Orion than in its surroundings by a factor of ~1.7 (refs 13, 14).

Later, we argue that the conversion from CO to H_2 depends on the metallicity (in particular, we take α as proportional to M^{-1}); to be consistent we must adopt the same procedure with Orion to derive from its α-value the corresponding average local value α_L. Because metallicities have not been determined for other galactic molecular clouds (as distinct from H II regions in the inner Galaxy which are used to give the so-called metallicity gradient), we adopt a local average value of α higher than α/M but lower than α. Accordingly, $\alpha_L = 2.7$ is used here as a compromise.

Other estimates of α locally

The Virial Theorem can be used to give estimates of cloud masses (strictly for clouds in equilibrium). Application to the local clouds surveyed by Stark and Blitz[15], after adopting intracloud velocities for the optically thin ^{13}CO line ($\simeq 1.5$ km s^{-1} half-width half maximum), yields $\alpha_L \simeq 2$. Direct determination of the column density of ^{12}CO from an optically thin transition in Orion[16] gives an upper limit to $N(H_2)$, implying $\alpha < 3.5$, if $^{12}CO/^{13}CO$ is $\simeq 40$, as often assumed. Another important result of this observation is that the velocity dispersion is $\lesssim 1.5$ km s^{-1}, a small value leading again to a small virial mass and a small α. Taken together, the evidence indicates that $2 < \alpha_L < 4$, hence we adopt $\alpha_L = 2.7$.

Cosmic-ray gradient

We have demonstrated that the new value of α (2.7) is 0.37 times that used in deriving the surface density of H_2 (Fig. 1). We next examine cosmic-ray evidence for an even smaller α value in the inner Galaxy. Starting with the γ-ray method, we examine the evidence for cosmic-ray gradients, that is, systematic changes of I_{CR} with R. There is agreement amongst those who have examined the SAS II (ref. 17) and COS B (ref. 18) γ-ray results[10,19] that there is a fall-off of CR electron intensity in the outer Galaxy (Fig. 3).

There is an approximate agreement of I_{CR} with the surface density of supernova remnants (Fig. 3) at the lower energies. There are admittedly problems with the determination of I_{CR} in the inner Galaxy (because of uncertainty in the effect of allowing for the ubiquitous H_2 component), but the situation in the outer Galaxy is well established. Here, there is fall-off in I_{CR} (ref. 10) and it is probable on general grounds that there will be a corresponding increase in I_{CR} in the inner Galaxy. It would be surprising if this increase in I_{CR} did not occur to at

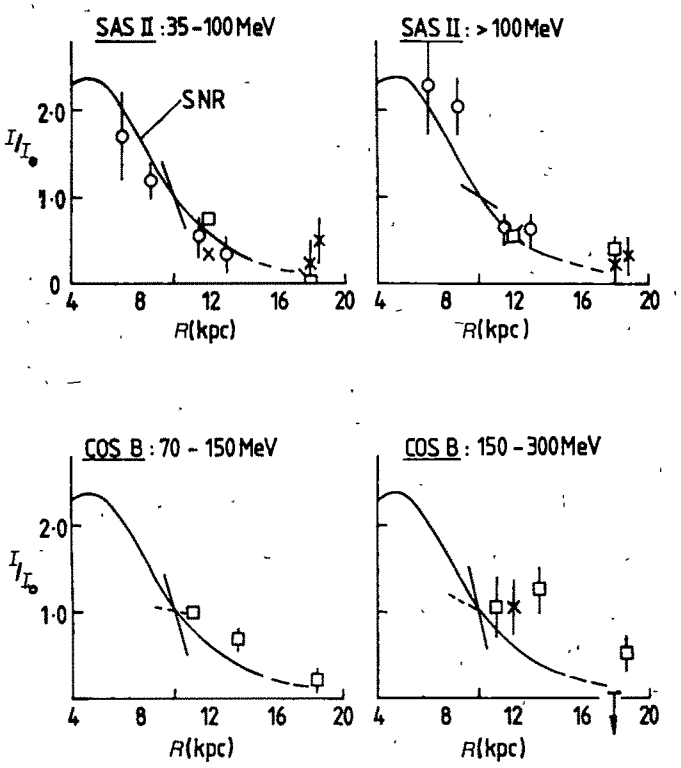


Fig. 3 Gradient of cosmic-ray intensity derived by various workers from the data of the SAS II experiment[17] and COS B[18]. The ordinate is the ratio of the cosmic-ray intensity to that locally, I/I_0. The SNR distribution[38] is shown as a probable distribution of the low energy cosmic rays responsible for the γ-rays. O, Data from Issa *et al.*[39]; □, data from Bloemen *et al.*[19]; ×, data from Bhat *et al.*[10,40]. The inclined lines through $R = 10$ kpc (the local position) refer to an analysis of γ-rays in the range $|b| = 11$–$19°$ (ref. 40); dotted inclined lines refer to an extreme Inverse Compton contribution; errors are statistical.

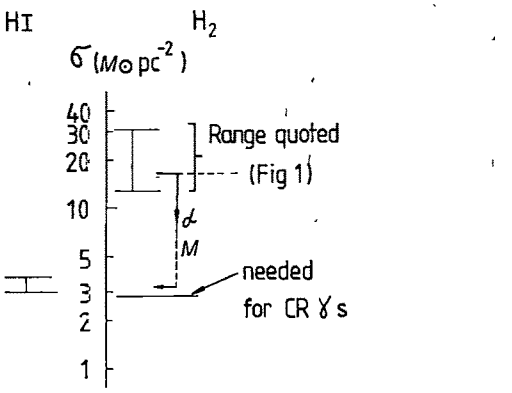


Fig. 4 Surface densities of H I and H_2 at $R = 6$ kpc. The range of quoted values from CO observations (using various α values) is indicated, as is the value from Fig. 1. The effect of reducing the surface density of H_2 by 0.37 times (to allow for the change in α) and by 0.5 (to allow for metallicity) is shown. Also indicated is the value needed to satisfy the cosmic γ-ray results.

least $R = 6$ kpc (all other indicators of galactic activity, such as H II regions, pulsars and H_2 surface density show similar increases). The effect of such an increase on the required surface density of all galactic gas is considered below.

Surface density of gas

The estimation of the surface density of gas at $R = 6$ kpc as required by the γ-ray data is straightfoward. Figure 4 gives the result. Taking the SSS value of $\sigma_{H_2}(R = 6\text{ kpc})$ as datum and 'correcting' to our new value of α (that is, $2.7/7.2 = 0.37$ times) yields a value of σ_{H_2} still too high for conformity with the γ-ray results; a further reduction is required. Therefore, we investigate the metallicity in the ISM and introduce a 'metallicity correction'[20,21]. Briefly, the O/H ratio in the ISM increases towards the Galactic Centre (GC) and we consider this to cause an increase in the $^{12}CO/H_2$ conversion, the factor of increase amounting to two at $R = 6$ kpc and ~3–5 at the GC. Applying the factor two, there is now a reduction in $\sigma_{H_2}(R = 6\text{ kpc})$ by a factor $0.37 \times 0.5 = 0.18$ of the SSS value, in agreement with the γ-ray expectation.

The use of the metallicity correction is controversial; it is argued against in ref. 1. The arguments for the correction are described in ref. 21 and further evidence is obtained from more detailed studies of interstellar chemistry described below. Higher temperatures and probable greater shock density in the inner Galaxy may augment or simulate a metallicity dependence.

Interstellar chemistry

We investigate the relationship between $N(^{12}CO)$ (the apparent column density of ^{12}CO), the actual value of N_{H_2} and the metallicity, characterized by the O/H ratio. We have assumed here that α is proportional to M^{-1} and we seek support for this relationship from arguments of interstellar chemistry. Comprehensive calculations have been made recently (D. A. Williams and T. Millar, personal communication and ref. 22).

Starting with the important optically-thin ^{13}CO component and limiting the argument to spherical inert clouds of mass $M_C(\sim 10^4\ M_\odot)$, the column density along a diameter divided by N_{H_2} varies approximately as $M^{1/2}$ (specifically for $T = 10$ K, gas density $n = 600$ nuclei cm^{-3}), that is, α is proportional to $M^{-1/2}$. The result is very similar for all densities in the range $200 < n < 1{,}800$ cm^{-3} and does not depend on cloud mass for $3 \times 10^3 < M_C < 10^5\ M_\odot$. The dependence on the ultraviolet intensity and the cosmic-ray heating rate is slight.

The results with ^{12}CO are very similar, although because ^{12}CO is optically thick, the detected signal in practice would not depend necessarily on M in the same way. It is here, however, that we return to the fact that over most of the Galaxy there is proportionality between the strengths of the ^{12}CO and ^{13}CO signals; we interpret this in terms of turbulence in the clouds and associated density variations such that velocity smearing allows ^{12}CO to be detected over wide regions of clouds. The result is that, although the premise of large uniform clouds (on which the chemistry calculations were made) breaks down, it seems to be in the sense of increasing the metallicity rather than reducing it. More sophisticated cloud modelling is necessary, but the best estimate so far is that α is approximately proportional to M^{-1}.

Inner Galaxy α estimates

There are other ways of estimating α at $R \simeq 6$ kpc, notably involving the virial mass, X-ray and infrared absorption, which are summarized here and discussed elsewhere in detail.

The virial masses are low when velocity dispersions for optically thin lines are used and we again have $\alpha \simeq 2$. The X-ray technique, which measures essentially the column density of atoms such as O, was first used with success by Blitz and Shu[20]. We have updated the treatment using new observations, with the result that $\alpha \simeq 1$ for $R \simeq 6$ kpc. Infrared observations give a complementary view; using the extinction data of Eaton *et al.*[23] we again find $\alpha \simeq 1$. The dust/gas ratio is probably lower by a factor of two in the region $R \simeq 6$ kpc (ref. 24) and applying this factor increases α to two.

The range of the α-values is 2 (virial), 1 or 2 (infrared) or 1 (X-ray), consistent with the γ-ray result, where $\alpha \simeq 1.4$.

Galactic Centre

Indirect evidence about the $CO \rightarrow H_2$ calibration comes from the claim by millimetre wave astronomers for considerable masses of H_2 within ~0.4 kpc of the GC on the basis of high

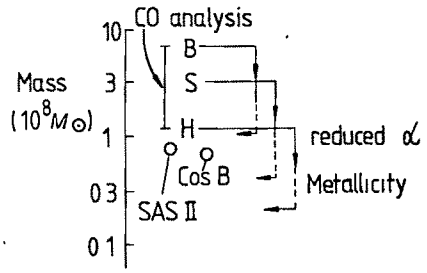


Fig. 5 Mass of gas within 400 pc of the Galactic Centre. The range of values corresponds to different CO analyses: B, data from Bania[41]; S, from Sanders *et al.*[1]; H, data from Heiligman[42]. Using our own α and the metallicity correction ($M = 3$) gives the reduced values shown by the arrows. Circles represent masses derived from the SAS II and COS B data assuming that the CR intensity at the Galactic Centre is the same as that locally (that is, $F = 1$). Adopting the mean of the reduced mass estimates would indicate $F \simeq 2$, a reasonable result.

$\int T(^{12}\mathrm{CO})\,\mathrm{d}V$ values in that direction. Several estimates have been made (Fig. 5).

For the γ-ray fluxes from the GC region, we follow the recent analysis of Houston and Wolfendale[25], who derived a GC 'source' flux of 2.3×10^{-6} cm^{-2} s^{-1} for $E > 100$ MeV for SAS II. For COS B, we identify the source 2CG359−00 from the catalogue of Hermsen[26] with the GC; this has a flux above the same energy of 1.8×10^{-6} cm^{-2} s^{-1}.

The problem now is the expected magnitude of I_{CR} in the GC region. A detailed study by Wolfendale and Worrall[27] shows that a self consistent model can be made in which the production rate of CR per unit mass of gas is approximately the same at the GC and locally (and is thus $\simeq 100\times$ greater than locally per unit volume at the GC). In the model, there is agreement with radio synchrotron data in showing that the ambient I_{CR} at the GC is $\sim 10\times$ that near the Sun. If this factor were used, we would expect a GC mass of $\simeq 0.07 \times 10^8\ M_\odot$, a very low value. As a lower limit to I_{CR} at the GC the local value can be used (that is $F = 1$), resulting in a mass of $\simeq 0.7 \times 10^8\ M_\odot$. The masses shown in Fig. 5 use $F = 1$.

The effect of applying the new α and the metallicity correction (a reduction of at least $M = 3$ at the GC) can now be examined. Figure 5 shows the new CO-implied masses which are now 'reasonable' in that they corespond to a modest CR enhancement of intensity ($F \simeq 2$). With the higher metallicity factor $M = 5$, implied by extrapolating the O/H gradient of -0.07 kpc^{-1} back to the GC, the mass would be correspondingly lower and $F \simeq 4$, a still reasonable enhancement.

Blitz *et al.*[28] have recently made a similar analysis and concluded that a very small α at the GC is probable. The main value of the present analysis of the GC is that it lends support to the presence of a metallicity effect (or perhaps shocks and temperature effects simulating it) and thereby to the correctness of our α-values in the inner Galaxy. (Blitz *et al.*[28] also make important comments about 'time variability' of the CO results; that is, the presence of systematic errors between different instruments and sets of measurements.)

Discussion

We have derived an independent estimate of the magnitude of the surface density of gas in the Galaxy as a function of galactocentric radius using γ-ray astronomy and supported by other techniques. Figure 1 shows that our estimate, $H_2(\gamma)$, is considerably lower than the 'conventional' estimate, from ref. 1, an estimate made using the usual procedure of relating $\int T\,\mathrm{d}V$ for the 2.6-mm CO line to the column density of H_2. Although we argue that the new estimate is reliable, it also contains assumptions; therefore, we have examined the 'conventional' method and, using the basic data on CO, made our own estimate of the surface density of the molecular gas.

The basic results[1] for $\int T\,\mathrm{d}V$ versus longitude have been taken, together with our own unfolding, to give the radial distribution of CO. Adopting radial symmetry, the result is CO surface densities ~60–80% of those given by SSS. This discrepancy presumably relates to differing assumptions in allowing for the clumpy nature of the gas distribution.

The important question of the CO → H_2 conversion has been examined in detail. Dickman's[29] results of $\int T(^{13}\mathrm{CO})\,\mathrm{d}V$ against visual extinction A_V for local clouds, and similar data for Taurus[30], have been examined and, as found by SSS, there is evidence for a saturation of $\int T\,\mathrm{d}V$ versus A_V. ($\int T\,\mathrm{d}V$ is proportional to $A_V^{0.5}$, approximately fitting a power law to the whole range in ref. 1, but we can have $\int T\,\mathrm{d}V$ proportional to A_V up to values of 6 for A_V for Taurus at least, with a flattening above.) It is conventional to follow Bohlin *et al.*[31] and adopt a linear relation of N_{H_2} versus A_V ($N_{H_2} = 0.95 \times 10^{21}\ A_V$ molecules cm^{-2}, although this relation has only been verified observationally for $A_V < 2$, clearly another region of uncertainty). The crucial questions concern the effective mean value of $\int T\,\mathrm{d}V$ for the GMC, where on the $A_V^{0.5}$ plot are we situated and the corresponding α value. These questions are examined in detail below.

For the Orion molecular clouds the 'median mass' $\int T(^{12}\mathrm{CO})\mathrm{d}V$ value (half the cloud mass is within and half without) is ~11 K km s^{-1} and 2 K km s^{-1} for ^{13}CO. Such a value is well on the linear part of the $\int T(^{13}\mathrm{CO})\,\mathrm{d}V$ against A_V relation.

Inspection of the data on Dickman's[29] clouds, five of which are completely mapped, indicates that the mean value of A_V is ~2.5 and the median $\int T(^{13}\mathrm{CO})\,\mathrm{d}V \sim 4$ K km s^{-1}. It is immediately apparent that with such a low value of $\int T(^{13}\mathrm{CO})\,\mathrm{d}V$, the derived α value will be low; specifically 3.0 for the five fully mapped clouds, very close to our earlier γ-ray value of 2.7 for the local region.

There is evidence that the 'clouds' detected on the galactic plane in the inner Galaxy have high values of $\int T(^{13}\mathrm{CO})\,\mathrm{d}V$ (for example, ref. 32, 7.2 K km s^{-1}, used in ref. 1), although other data[33] give values of only ~3 K km s^{-1}. We consider that many of the so-called clouds are almost certainly conglomerates of smaller clouds, each of the latter probably resembling those seen locally (Orion, Taurus). If this is so, we are not on the saturated part of $\int T\,\mathrm{d}V$ versus A_V, which corresponds for local clouds to those rare passages through very dense regions, but, usually to low values of $\int T\,\mathrm{d}V$, on the linear part. For example, with passage through three clouds, the operative value of $\int T\,\mathrm{d}V$ is $7.2/3 = 2.4$ K km s^{-1}. This contention is supported by data on identified clouds of large radius[34], indicating that the mean density (molecules cm^{-3}) of the clouds falls as their linear dimension increases; this is what we would expect if loose conglomeration were occurring. The point now is that the separate parts of the conglomerate have different velocities and the constituent $\int T\,\mathrm{d}V$ values are additive.

Using the analysis presented here, we can derive the 'new' mass of H_2 in the inner Galaxy (corresponding to $H_2(\gamma)$ in Fig. 1). Neglecting the region $R < 1$ kpc, the value is 6×10^8 M, compared with 8×10^8 M for H I.

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1 Sanders, D B, Solomon, P M & Scoville, N Z *Astrophys J* **276**, 182–203 (1984)
2 Fichtel, C E & Kniffen, D A *Astr Astrophys* **134**, 13–23 (1983)
3 Houston, B P. & Wolfendale, A W *J Phys* G**10**, 1587–1598 (1984)
4 Houston, B P thesis, Univ Durham (1984)
5 Houston, B P & Wolfendale, A W *J Phys* G (in the press)
6 Skilling, J & Strong, A W *Astr Astrophys* **53**, 253–258 (1976)
7 Arnaud, K *et al Mon Not R. astr Soc.* **201**, 745–758 (1982).
8 Gordon, M. A & Burton, W B *Astrophys J* **208**, 346–353 (1976)
9 Li, Ti pei, Riley, P A & Wolfendale, A W *Mon Not R. astr Soc.* **203**, 87–102 (1982)
10 Bhat, C L, Mayer, C J & Wolfendale, A W *Astr Astrophys* **140**, 284–287 (1984)
11 Issa, M R & Wolfendale, A W *Nature* **292**, 430–433 (1981)
12 Issa, M R & Wolfendale, A W *J Phys* G**7**, L187–L191 (1981)
13 Pagel, B E J & Edmunds, M G *A Rev Astr Astrophys* **19**, 77–113 (1981)
14 Peimbert, M *Ann N Y Acad Sci* **395**, 24–31 (1982)
15 Stark, A A & Blitz, L *Astrophys J* **225**, L15–19 (1978)
16 Black, J H & Willner, S P *Astrophys J* **279**, 673–678 (1984)
17. Fichtel, C E *et al NASA Tech Mem X-662-76-198* (GSFC Greenbelt, 1976)
18 Mayer-Hasselwander, H A *et al Astr Astrophys* **105**, 164–175 (1982)
19 Bloemen, J E G M *et al Astr Astrophys* **135**, 12–22 (1984)
20 Blitz, L & Shu, F H *Astrophys J* **238**, 148–157 (1980)
21 Li, T P, Riley, P A & Wolfendale, A W *J Phys nucl Phys* G**8**, 1141–1154 (1982)

22 Williams, D A & Hartquist, T W *Mon Not R astr Soc* **210**, 141-145 (1984)
23 Eaton, N, Adams, D J & Giles, A P *Mon Not R. astr Soc* **208**, 241-251 (1984)
24 Bhat, C L, Mayer, C J & Wolfendale, A W (in preparation)
25 Houston, B P & Wolfendale, A W *Astr Astrophys* **126**, 22-30 (1983)
26 Hermsen, P *Phil Trans R Soc* A301, 519-521 (1981)
27 Wolfendale, A W & Worrall, D M *Astr Astrophys* **60**, 165-170 (1977)
28 Blitz, L, Bloemen, J B G M, Hermsen, W & Bania, T M., *Astr Astrophys* (in the press)
29 Dickman, R L *Astrophys J Suppl* **37**, 407-427 (1978)
30 Frerking, M A, Langer, W D & Wilson, R W *Astrophys J* **262**, 590-605 (1982)
31 Bohlin, R C, Savage, B D & Drake, J F *Astrophys J* **224**, 132-142 (1978)
32 Liszt, H S, Xiang, D & Burton, W B *Astrophys J* **249**, 532-549 (1981)
33 Stark, A A thesis, Univ Princeton (1979)
34 Dame, T M. thesis, Goddard Inst for Space Studies, New York (1984)
35 Solomon, P M & Sanders, D B in *Giant Molecular Clouds in the Galaxy* (eds Solomon, P M & Edmunds, M G) 41-73 (Pergamon, Oxford, 1980)
36 Weaver, H & Williams, D R W *Astr astrophys Suppl* **8**, 1-503 (1973)
37 Kutner, M L, Tucker, K D, Chin, G & Thaddeus, P *Astrophys J* **215**, 521-528 (1977)
38 Kodaira, K *Publ astr Soc Jap* **26**, 255-265 (1974)
39 Issa, M R, Riley, P A, Strong, A W & Wolfendale, A W *J Phys G* **7**, 973-994 (1981)
40 Bhat, C L, Houston, B P, Issa, M R, Mayer, C J & Wolfendale, A W *Proc Abingdon ISM Conf* 39-54 (1984)
41 Bania, T M *Astrophys J* **216**, 381-403 (1977)
42 Heiligman, G M thesis, Univ. Princeton (1982)

LETTERS TO NATURE

Acceleration of cosmic rays in the Loop I 'supernova remnant'?

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Attempts are being made to establish the origin of cosmic rays using the γ-ray method, that is, by dividing γ-ray intensity by gas-column density to chart the cosmic-ray intensity. Here we present evidence from satellite data on γ rays and from new results on the distribution of gas in the interstellar medium (ISM) for an enhancement of electron and proton intensity in the Loop I supernova remnant (SNR). There follows from this a straightforward explanation for the origin of the bulk of the low-energy cosmic radiation.

Here, we describe a study of the secondary γ rays in the 100-MeV region, generated by the interactions of cosmic rays (protons of 1-10 GeV and electrons of several hundred MeV) with the ISM. It is generally considered[1,2] that most of the γ rays detected in the satellite experiments are, in fact, generated by these interactions rather than by unresolved discrete sources, particularly at the galactic latitudes, $|b| > 10°$, considered here.

Early work by Dodds *et al.*[3] using SAS II data[4] gave evidence (subsequently confirmed[5,6]) for a gradient of cosmic-ray intensity in the outer galaxy, indicative of a galactic origin for the mixed beam of relevant electrons and protons. To distinguish between the relative contributions of protons and electrons, however, is not a trivial problem and, although it is now generally agreed that there is a gradient for electrons[5,6], the situation for protons is debatable[6,7]. (In fact, acceptance of the arguments presented here would resolve the debate; we shall argue that the strong local gradient[7] will have an important contribution from the Loop.)

That there is a strong correlation of the γ-ray flux with the intensity of synchrotron radiation, both in and out of the plane is well known[8,9]. Prominent features of the synchrotron map out of the plane are a number of Loops of which the most prominent is Loop I. This has been identified by Berkhuijsen *et al.*[10] with a SNR centred on a point ~130 pc away, with diameter ~230 pc and having central coordinates $l = 329°$, $b = +17.5°$. The corresponding position for the supernova was 40 pc above the plane and the bulk of the shock front is still in the gas disk; the Loop is prominent between latitudes −30°-+80° and longitudes 260°-60°.

It is natural to search for a specific correlation of the γ-ray flux with this Loop to support a popular hypothesis[11-15] that the supernova is the seat of cosmic-ray acceleration. We mention particularly the work of Blandford and Cowie[15], who have not only considered the cosmic-ray energetics of radio loops but have made specific predictions of γ-ray emissivities.

Concerning γ rays, there are data from the SAS II experiment[16] and, for each of a number of latitude bands (Fig. 1), we have determined the dependence of γ-ray intensity on longitude. The expected γ-ray intensity for the same conditions has been derived assuming a constant cosmic-ray intensity and adopting a new estimate of the column density of gas.

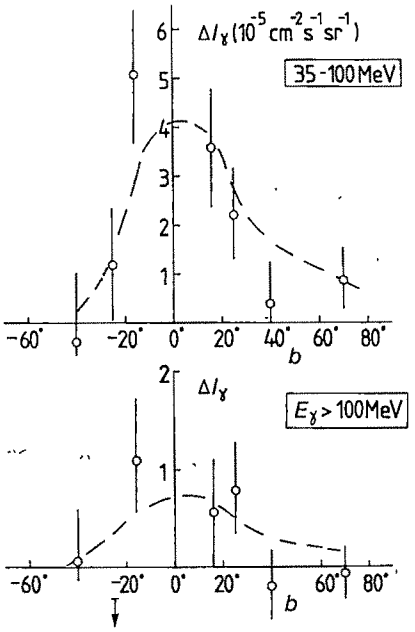


Fig. 1 The excess γ-ray intensity associated with Loop I as a function of galactic latitude. The basic γ-ray data are from the SAS II experiment[16] ΔI_γ is the difference between observed and expected γ-ray intensity for the Loop region minus the same quantity outside the Loop. The smooth curve is the corresponding T_{408}, averaged in the same way, normalized in height to observation. The particles generating the γ rays are almost entirely electrons in the lower energy band. At higher energies protons generate about 60% of the γ rays

The column density of gas (N) comprises two components—HI, N_{HI} and H_2, N_{H_2}. The former is well known[17-19], but the latter is uncertain and it is on the choice for N_{H_2} that the present work rests; thus, if we have seriously underestimated N_{H_2}, our conclusions will be invalid. Our method consists of using galaxy counts[20] to give N_{H_2} for those regions where CO observations are not available and calibrating the galaxy-count column density against N_{H_2} in the region where CO data are available (l: 5°-35°; b. 10°-20°; ref. 21). For the longitude range above $l = 210°$, where galaxy counts cease, we adopt interstellar reddening data[8,9]. As an indication of the importance of H_2 in the lowest latitude range considered (12.8°-19.5°), the adopted average N_{H_2} is ~25% that for N_{HI}. Other spot measurements using different techniques in the l,b range considered also give very small N_{H_2} values[22,23]. For latitudes outside $|b| = 20°$, we follow previous work[24,25], neglecting N_{H_2} altogether. The emissivity values used to derive the predicted γ-ray intensities are $q/4\pi = 3.7 \times 10^{-26}$ and 2.6×10^{-26} atom^{-1} s^{-1} sr^{-1} for 35-100

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and >100 MeV respectively. (In fact the absolute values are not very important because of the subtraction technique used.)

The procedure is now to determine the excess of the observed γ-ray intensity relative to that expected as a function of longitude, $I_e(l)$, both inside the loop region, $I_{e,L}$, and outside it, $I_{e,o}$, and to determine $\Delta I_\gamma = I_{e,L} - I_{e,o}$, representing the excess contributed by the Loop (the longitude range embraced by the Loop is $l = 260° \rightarrow 60°$ over the relevant latitudes). Figure 1 shows the Loop contribution as a function of latitude. A similar analysis has been made for the COS B data[20] in the latitude range $|b| = 11° \rightarrow 19°$, but, unfortunately, no COS B data are yet available for $|b| > 19°$. Figure 2 gives a comparison of the Loop contributions for the positive (N) and negative (S) latitude ranges ($11° \rightarrow 19°$) (correcting the SAS II values to the same b-range).

There is no question of the γ-ray excess being constant over the SNR; in fact, it peaks just inside the well known radio-continuum North Polar Spur, but, insofar as we wish to determine the energetics of the remnant, the analysis is made in terms of averages over the object. Figure 1 contains a plot of ΔT_{408} (T_{408} is the antenna temperature of the radio emission at 408 MHz) against latitude from which the general correlation can be seen. It is obvious that there is clear evidence for a Loop contribution, particularly for the 35–100 MeV data.

Discounting the possibility that a dense gas cloud in the direction of the Loop has been missed, the most probable alternative explanation to cosmic-ray acceleration in a SNR is that we are dealing with inverse Compton (IC) interactions of electrons with an enhanced radiation field in the vicinity of the Loop. Using the analysis of Riley and Wolfendale[8], it seems unlikely that >50% of the Loop contribution can be attributed to IC, at any energy; Bloemen's work would give an even smaller IC contribution (J. B. G. M. Bloeman, personal communication). It is reassuring that the COS B excess is present at all energies and; given that cosmic-ray protons predominate as primaries of the γ rays in the highest energy region, this is the best evidence for an excess of protons in the Loop.

We turn now to questions of enhanced cosmic-ray intensities and energetics, starting with the electron component. That the Loop is actually seen in synchrotron radiation shows that there is an enhanced cosmic-ray electron intensity and/or an increased magnetic field intensity (the electron energy being ~3 GeV for typical ISM fields). Surprisingly perhaps the magnetic field seems not to be enhanced[26] and thus the electron intensity must be increased considerably. Heiles *et al.*[26] estimate an increase by a factor $F_e \sim 20$ in the radio-continuum North Polar Spur. Averaging over the whole SNR gives an electron enhancement factor $F_{e,r} \sim 5$–10.

Figure 1 ($E_\gamma = 35$–100 MeV) can be used to estimate F_e for the initiating electrons for the γ rays ($\langle E_e \rangle \sim 200$ MeV). Using the model of Blandford and Cowie[15] with a mean density of 0.4 cm^{-3} (0.2 cm^{-3} in the hot ISM and 0.2 cm^{-3} for the average contribution of cold sub-clouds) the average enhancement factor is $F_{e,\gamma} \sim 6$, in good agreement with $F_{e,r}$.

We now consider the ambient energy density of the cosmic-ray electron component needed to calculate the electron energy residing in the remnant. The magnitude seems to depend critically on the lower limit to the energy, unlike the proton case, because of the need for a steep electron spectrum below 1 GeV to explain the ambient γ-ray and radio spectra. Adopting the interstellar electron spectrum of Strong and Wolfendale[27], the energy densities in electrons are 2.3×10^{-2} eV cm^{-3} above 100 MeV and 3×10^{-3} eV cm^{-3} above 1 GeV, to be compared with ~0.5 eV cm^{-3} above 100 MeV and ~0.4 eV cm^{-3} above 1 GeV for protons.

Adopting the 100-MeV limit and $F_{e,\gamma} = 6$, the total energy in electrons in the remnant is $\sim 2 \times 10^{49}$ erg. Turning now to protons, the mean value for SAS II and COS B is $F_{p,\gamma} \sim 4$ and the corresponding energy is 2×10^{50} erg. Considering the problem of the IC contribution, it is best to reduce these values by a factor of two, to $E_e \sim 10^{49}$ erg and $E_p \sim 10^{50}$ erg and attach an uncertainty of a factor of three to each.

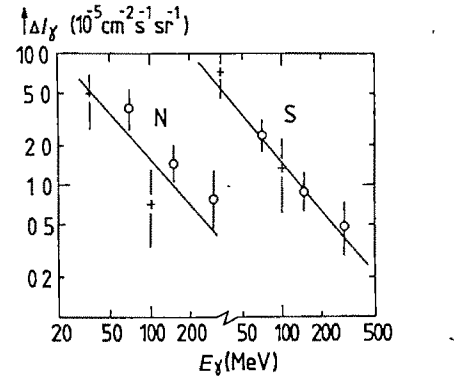


Fig. 2 The excess γ-ray intensity defined in Fig. 1 as a function of threshold energy. The latitude ranges are: N 11°→19°, S −11°→−19°. The circles refer to COS B data and the crosses to SAS II. The spectral shape is not dissimilar to the average spectrum of galactic γ rays. There appears to be a significant excess for the highest energy γ rays, these quanta being generated very largely by cosmic-ray protons

We can now make a comparison with the predictions of Blandford and Cowie[15]; their average γ-ray surface brightness, above 100 MeV, of 7×10^{-6} cm^{-2} s^{-1} sr^{-1} is very similar to that in Fig. 2. Concerning the energy content of the Loop, our estimate is $\sim 10^{50}$ erg for protons and $\sim 10^{49}$ erg for electrons, again close to estimates based on the Blandford and Cowie model for a supernova of initial energy 10^{51} erg.

We can now return to the important question of the acceleration of protons; unlike electrons, for which there is the synchrotron enhancement, the γ-ray results alone provide supporting evidence. The argument in favour of accelerated protons is demonstrated by the higher energy band in Fig. 1 (protons probably give ~60% of the γ rays) and the two highest energy bands in Fig. 2, particularly that for $E_\gamma > 300$ MeV, where we expect at least 80% to have been produced by protons. An important feature here is that the correlation between I_γ (> 300 MeV) and T_{408} is singularly strong, thus confirming that the protons are being accelerated along with the electrons. That we find approximate agreement between $F_{e,r}$ and $F_{e,\gamma}$ suggests that our estimates of gas column density are not seriously in error.

The implications of the results for the origin of cosmic rays are obvious, the most important feature being that the efficiency for conversion of supernova energy into cosmic-ray protons is very high. For Loop I we have presently about 10% of the initial energy in protons, which presumably will soon be released into the ISM. The energy requirement for cosmic radiation in the Galaxy can be calculated as follows. With a galactic volume for cosmic rays of 8×10^{67} cm^3, a lifetime for the particles of 2×10^7 yr and an ambient energy density of 0.5 eV cm^{-3}, the cosmic-ray energy input is $\sim 10^{41}$ erg s^{-1}. Adopting a supernova rate of 1 per 30 yr and total energy per supernova of 10^{51} erg, the efficiency required for generation of cosmic radiation at the moment of release into the ISM is simply 10%, that is, the value we estimate for Loop I. Such agreement is surely fortuitous in view of the many uncertainties involved, but it does demonstrate the cogency of the entire argument. We are currently looking for the expected cosmic-ray enhancements in other SNR to attempt to confirm our conclusions.

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1 Fichtel, C E & Kniffen, D A *Astr Astrophys.* 134, 13-23 (1984)
2 Houston, B P & Wolfendale, A W *Astr. Astrophys* 126, 22-30 (1983), *J Phys G* (in the press).
3 Dodds, D, Strong, A W & Wolfendale, A W *Mon Not. R. astr Soc.* 171, 569-577 (1975).
4 Fichtel, C E *et al. Goddard Space Flight Center* X-662, 74-304 (1974); *Astrophys J* 198, 163-182 (1975)
5. Issa, M R, Riley, P A, Strong, A W & Wolfendale, A W *J Phys* G7, 973-994 (1981)
6 Bloemen, J B G M *et al Astr Astrophys* 135, 12-22 (1984).
7 Bhat, C. L., Mayer, C J & Wolfendale, A W *Astr Astrophys* 140, 284-287 (1984)
8. Riley, P A. & Wolfendale, A W *J Phys. G* 10, 1149-1161 (1984)
9 Haslam, C G T, Kearsey, S, Osborne, J. L, Phillipps, S & Stoffel, H *Nature* 289, 470-473 (1983)

10 Berkhuijsen, E M, Hasam, C G T & Salter, C J *Astr Astrophys* **14**, 252-262 (1971)
11 Zwicky, F *Proc natn Acad Sci* **25**, 338-344 (1939)
12 Ginzburg, V L & Syrovatsky, S I *The Origin of Cosmic Rays* (Pergamon, Oxford, 1964)
13 Axford, W I *Ann N Y Acad Sci* **375**, 297-313 (1981)
14 Drury, L O'C & Volk, H J *Astrophys J* **248**, 344-351 (1981)
15 Blandford, R D & Cowie, L L *Astrophys J* **260**, 625-634 (1982)
16 Fichtel, C E *et al NASA Tech Memo* 79650 (1978)
17 Weaver, H & Williams, R W *Astr Astrophys Suppl* **8**, 1-503 (1973)
18 Heiles, C & Habing, H J *Astr Astrophys Suppl* **14**, 1-50 (1974)
19 Heiles, C & Cleary, M N *Aust J Phys astrophys Suppl* **47**, 1-58 (1979)
20 Strong, A W *et al Astr Astrophys* **115**, 404-412 (1982)
21 Lebrun, F & Huang, Y L *Astrophys J* **281**, 634-638 (1984)
22 Bohlin, R C, Savage, B D & Drake, J F *Astrophys J* **224**, 132-142 (1978)
23 Blitz, L, Fich, M & Stark, A A *Astrophys J Suppl* **49**, 183-206 (1982)
24 Dewey, R J, Uson, J M, Weisberg, J M, Wilkinson, D T & Stark, A A *Astr J* **88**, 1832-1834 (1983)
25 Blitz, L, Magnani, L & Mundy, L *Astrophys J* (in the press)
26 Heiles, C, Chu, Yuo-Hua, Reynolds, R J, Yegingil, I & Troland, T H *Astrophys J* **242**, 533-540 (1980)
27 Strong, A W & Wolfendale, A W *J Phys. G* **4**, 1793-1807 (1978)

Terrestrial impactors at geological boundary events: comets or asteroids?

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Evidence has been presented[1,2] for a 26–28-Myr periodicity in both the terrestrial extinction record and the age of large well-dated impact craters on the Earth. A cometary source controlled by perturbations associated with either the Solar System's galactic z-motion, which has a 33-Myr half-period, or an unseen solar companion star in a 26–28-Myr orbit has been suggested[3–5] for the impacts. Identifications of meteoritic material in the impact melts of the craters[6] used to demonstrate the periodicity, however, are indicative of highly differentiated impactors, which are more representative of asteroids than of comets. Clustering of crater ages at some dates may be indicative of random comet showers, some associated with extinctions and some not. The source of asteroidal impactors with a ~28-Myr period remains unknown and casts doubt on the entire periodicity argument.

The cause of biological extinction events at geological boundaries has been much studied recently, especially since the detection of extraterrestrial material at the Cretaceous–Tertiary (K–T) boundary[7]. Among the extinction triggering mechanisms suggested are passage of the Solar System through interstellar dust clouds, asteroid and comet impacts, geomagnetic field reversals, nearby supernova explosions and exposure of the Earth to intense galactic cosmic rays. The impact arguments have gained wide acceptance, at least for the well-studied K–T event where shocked quartz grains (presumably impact ejecta) have been found[8], in addition to the large global siderophile anomalies.

The 28-Myr periodicity in the crater record is based on 16 craters with diameters >5 km and ages whose probable error is <20 Myr[2,9]. These craters are listed in Table 1 along with their diameters and ages. For eight of the sixteen craters, identifications of the impacting body composition have been made (Table 1) based on meteorite siderophile signatures in the impact melt[6]; in some cases the identification of impactor types is not completely certain.

Of the eight identified impactor compositions, six are differentiated or highly differentiated meteorite types. Formation of iron, stony and achondrite meteorites all require melting and differentiation in fairly large parent bodies. Ordinary chondrites may or may not be partially melted, but are depleted in volatiles as compared with the more-primitive carbonaceous chondrites. Only the impactors at Wanapitei, Canada at 37±2 Myr and Lappajarvi, Finland at 77±4 Myr are primitive undifferentiated meteorite types. Interestingly, Lappajarvi is the only crater in the list (except for the youngest one, of age 7 Myr) which is not identified with a periodic impact[2]; it falls almost exactly between the impact cycles at ~65 and 95 Myr.

Table 1 Periodic terrestrial craters

Location	Diameter* (km)	Age (Myr)*	Projectile type†
Karla, USSR	10	7±4	
Haughton, Canada	20	13±11	
Ries, Germany	24	14.8±0.7	Achondrite (?)
Mistastin, Labrador	28	38±4	Iron or achondrite (?)
Wanapitei, Ontario	8.5	37±2	Chondrite (C1, C2, LL)
Popigai, Siberia	100	39±9	Iron
Lappajarvi, Finland	14	77±4	C-chondrite
Steen River, Alberta	25	95±7	
Boltysh, Ukraine	25	100±5	
Logoisk, USSR	17	100±20	
Mien Lake, Sweden	5	118±2	Stony (?)
Gosses Bluff, Australia	22	130±6	
Rochechouart, France	23	160±5	Iron (IIA) or chondrite (?)
Obolon, Ukraine	15	185±10	Iron
Puchezh-Katunki, USSR	80	183±3	
Manicougan, Quebec	70	210±4	

* Data from refs 2 and 6
† Data from refs 6 and 9

It is difficult to reconcile impact craters formed by highly differentiated meteorite types with a cometary source. Comets are believed to have formed[10] as primitive undifferentiated icy planetesimals in the region of the outer planets and beyond. The typical cometary nucleus is believed to be a heterogeneous mixture of icy volatiles (mostly water ice) and micron-sized dust particles, a few kilometres in diameter[11]. Because of their small size neither gravitational accretion energy nor internal radiogenic heating is sufficient to differentiate the nuclei[12], whose large formation distance from the sun and long-term storage in the Oort cloud precludes any subsequent possibility of extreme heating.

Interplanetary dust particles recovered by high-flying aircraft, which are presumed to have a cometary origin, seem to be agglomerations of interstellar dust grains and show no evidence of melting or differentiation[13]. Although comets may be a source for the very primitive carbonaceous chondrites, even those meteorites may be too processed to come from such a primitive source.

On the other hand, the differentiated meteorite types are believed to come from large parent bodies where gravitational and radiogenic heating is sufficient to have caused either partial or complete melting, or more modest-sized bodies where the heating has only been sufficient to drive off the more volatile elements and compounds and to cause partial melting of individual chondrite grains. Reflectance spectroscopy studies of meteorites and asteroids have shown many common analogues between the two, both in the main belt and among the known Earth-crossing asteroids (L. A. McFadden, M. J. Gaffey and T. B. McCord, personal communication). Thus, the data on impactor compositions at six of the seven periodic craters in the sample are more consistent with an asteroidal source for the impactors than with a cometary one.

Additionally, although no specific impact crater has been identified with the K–T extinction, Palme has found that the siderophile signature in the boundary clay "does not match the siderophile element pattern of unfractionated meteorites (chondrites)"[9]. An iron asteroid impactor has been suggested but is not a certainty.

It is difficult to imagine a dynamic mechanism that would supply asteroidal impactors to the Earth with a 26–28-Myr period. The typical dynamic half-life for bodies placed in Earth-crossing orbits is ~30 Myr. Impacts from any periodic injection of asteroids into Earth-crossing orbits would be scattered randomly in time over that period. What would be needed is a mechanism that both injected bodies into Earth-crossing orbits periodically and then destroyed them relatively quickly (in 1–5 Myr) if they were not swept up by the Earth.

If there is no dynamic mechanism for supplying asteroidal impactors with the suggested (or any) period, then one may

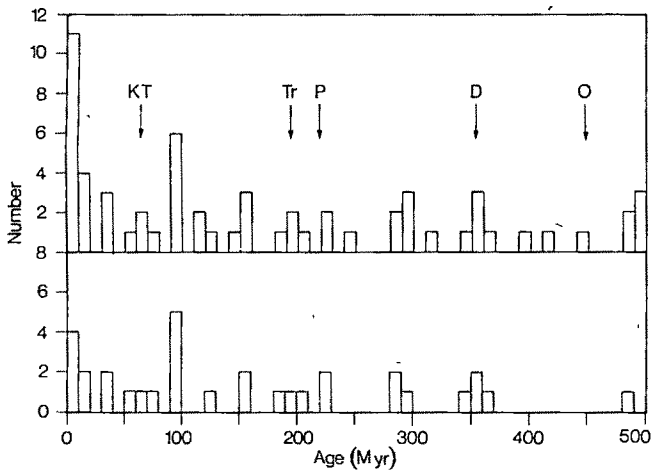


Fig. 1 Distribution of ages for dated craters on the Earth. Upper histogram is for all dated craters; lower histogram is for those with diameter ≥10 km. Arrows indicate the approximate times of the five major biological extinction events in the past 500 Myr.

suspect that the periodicity in the cratering and extinction records is not real but is a statistical fluke. Additionally, one may speculate that the extinction mechanism is not always impact related but also results from some other terrestrial or extraterrestrial event or process, such as the passage of the Solar System through a giant molecular cloud or close to a supernova explosion or an unusual period of terrestrial vulcanism.

Another question is, if there are no periodic comet showers from the inner Oort cloud, is there still evidence of random showers as suggested by Hills[14]? The distribution of ages of known impact craters on the Earth over the past 500 Myr is shown in Fig. 1. The upper histogram includes 61 craters listed by Grieve[6], without regard to the formal error on the age; the lower histogram includes only those 32 craters with diameters ≥10 km. Also shown are the approximate times of the five major extinctions in the past 500 Myr, as given by Sepkoski[15].

There seem to be non-random clusterings of craters at certain times in the Earth's past, and in some cases these may be correlated with recognized extinction events. In particular, the five craters at ~360 Myr BP (four of them ≥10 km in diameter) are close in age to the late Devonian (D) extinction event; an iridium anomaly was recently identified at this boundary[16]. Another grouping of five smaller craters at ~490 Myr BP may be associated with one of the lesser extinction events identified by Sepkoski, the Cambrian biomere events. On the other hand, a cluster of five craters at ~290 Myr BP (three, ≥10 km) is not associated with any recognized extinction and the late Ordovician (O) extinction at ~440 Myr BP has no substantial clustering of impacts simultaneous with it. The record is less clear for the Permian (P), Triassic (Tr) and K–T events where there is a more continuous distribution of large and small craters in time.

The crater clusterings associated with the late Devonian and Cambrian biomere events may be evidence of random cometary showers in the past, caused by random stars passing through the inner Oort cloud. A random star might be expected to pass within 10^4 AU of the sun, the approximate transition distance between the inner and outer Oort cloud, every 80 Myr[17]. Of the 10 craters possibly associated with these two events, only the 5-km crater at Saaksjarvi, Sweden has an identified impactor type, a stony-iron. The lack of identified impactors is largely attributable to a failure to search for them. Higher velocity impacts, however, tend to leave a smaller fraction of the meteoritic material in the impact melts[9], making the impactor type harder to identify. Cometary impacts are expected to be at average velocities of 29 km s^{-1} for short-period comets and 57 km s^{-1} for long-period comets, considerably greater than the 15–20 km s^{-1} expected for asteroids[18]. On the other hand, the crater clusterings may only be the result of random small-number statistics; more detailed analysis is clearly required.

It seems that there is not a simple one-to-one relationship between major asteroid and/or comet impacts, siderophile anomalies and biological extinction events on the Earth. Although impacts may be occasionally, or even often, the major triggering event, it is still possible that there are other types of events that may also trigger extinctions. Similarly, the secondary mechanism that actually causes the extinction, for example, long-term darkness, planetary cooling or atmospheric pollution, may vary from one extinction to another depending on the exact state of the planet and its biota at the time of the event. These questions remain open, awaiting new and better data for their resolution.

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1 Raup, D M. & Sepkoski, J J Jr *Proc. natn. Acad. Sci USA.* **81**, 805–805 (1984)
2 Alvarez, W & Muller, R. A *Nature* **308**, 718–720 (1984)
3. Whitmire, D P. & Jackson, A A Jr *Nature* **308**, 713–715 (1984)
4 Davis, M , Hut, P & Muller, R A *Nature* **308**, 715–717 (1984)
5 Rampino, M R & Stothers, R B *Nature* **308**, 709–712 (1984).
6 Grieve, R. A F *Geol. Soc. Am spec. Pap* **190**, 25–37 (1982)
7. Alvarez, L W., Alvarez, W , Asaro, F & Michel, H V *Science* **208**, 1095–1108 (1980).
8 Bohor, B F , Foord, E E , Modreski, P J & Triplehorn, D M *Science* **224**, 867–869 (1984)
9. Palme, H *Geol. Soc. Am. spec. Pap* **190**, 223–233 (1982)
10 Weissman, P. R. in *Protostars and Planets II* (ed Black D C) (University of Arizona Press, in the press)
11. Donn, B & Rahe, J in *Comets* (ed Wilkening, L L) 203–226 (University of Arizona Press, 1982)
12 Lewis, J S *Icarus* **15**, 174–185 (1971)
13 Fraundorf, P , Brownlee, D E & Walker, R M in *Comets* (ed Wilkening, L L) 383–409 (University of Arizona Press 1982)
14 Hills, J G *Astr J* **86**, 1730–1740 (1981)
15 Sepkoski, J J. Jr *Geol. Soc. Am spec. Pap* **190**, 283–289 (1982)
16 Playford, P E , McLaren, D J , Orth, C J , Gilmore, J. S & Goodfellow, W D *Science* **226**, 437–439 (1984)
17 Weissman, P. R. *Nature* **288**, 242–243 (1980)
18 Weissman, P R *Geol. Soc Am. spec. Pap* **190**, 15–24 (1982)

Timescale of natural annealing in radioactive minerals affects retardation of radiation-damage-induced leaching

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There is concern that if solidified nuclear waste is exposed to ground water, accelerated dissolution of radioactive waste nuclides will ensue because of the accumulation of radiation damage created by α decay. We report here the results of radioisotope measurements on ancient uranium- and thorium-rich minerals and find that 'self-annealing' strikingly reduces the rate of dissolution. That short-lived ^{228}Th is leached, whereas long-lived ^{230}Th and ^{234}U are not, sets a limit on the timescale of self-annealing; given a simple assumption, we estimate an annealing time of 15 kyr. Two further conclusions emerge. First, even though the overall structure of the minerals remains crystalline (inferred from X-ray diffraction), there must be localized submicroscopic loss of order because preferential leaching takes place. Second, the inference that damage fades and slows the leaching rate indicates that minerals in which such annealing occurs are better waste-storage media for minimizing loss of radionuclides by dissolution.

Safe storage of solid nuclear waste requires a matrix that will not be easily dissolved by ground water if a buried container is breached. Although waste-storage materials that have minute

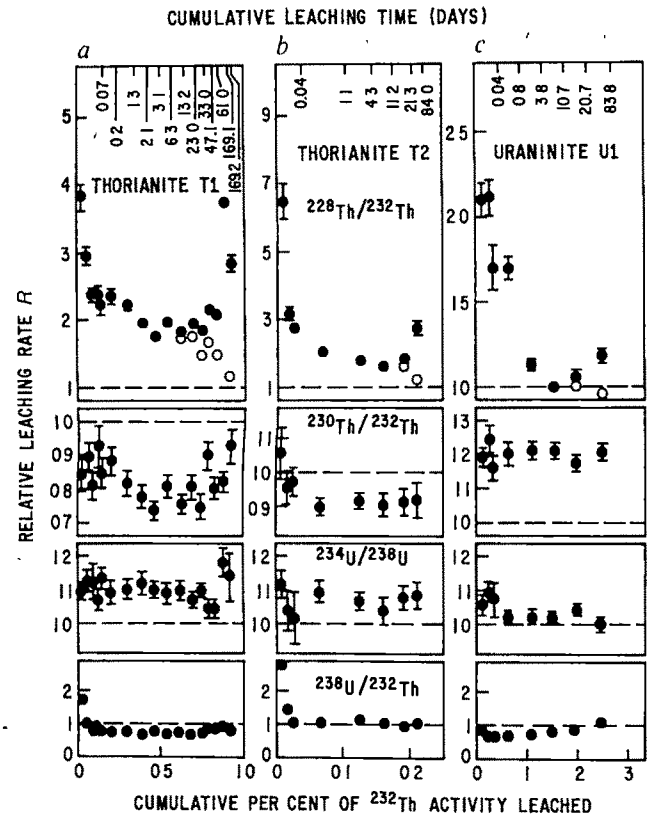


Fig. 1 Relative leaching rates R (solid circles) of uranium and thorium isotopes leached at 25 °C by a bicarbonate/carbonate solution from two thorianite specimens (*a*, *b*) and a uraninite (*c*). The value of R is the ratio of fractions of radioactivities leached. Values of $R(^{228}Th/^{232}Th) \gg 1$ show that the ^{228}Th lies in leachable radiation-damage sites, whereas values of $R(^{230}Th/^{232}Th)$ and $R(^{234}U/^{238}U)$ elevated by no more than 20% imply that the damage fades over considerably less time than the mean life of ^{234}U (3.5×10^5 yr). For the $^{228}Th/^{232}Th$ fractionation, the open circles give the lower limit of $R(^{228}Th/^{232}Th)$, assuming the ancestor nuclide of ^{228}Th, ^{228}Ra, is completely retained in the solid phase.

leaching rates are available, it is possible[1] that the radioactivity that is to be isolated will itself cause radiation damage and thus bring about greatly accelerated chemical attack by ground water. The major source of damage is expected to result from α decay of heavy elements, which produces heavy residual nuclei that recoil a distance of ~200 Å, displacing ~1,500 atoms[2].

The evidence for accelerated dissolution by such damage comes both from laboratory experiments and field observations. Individual α-recoiling nuclei can be implanted in a crystal or glass and then released by exposure to water[3,4], implying that the damaged region is dissolved to where the implanted atoms come to rest and that doses of implanted low-energy ions sufficiently intense that the clumps of diplaced atoms overlap, produce bulk dissolution to the depth of the implantation[5]. Ground water commonly leaches ^{234}U, in preference to ^{238}U, out of rocks that it permeates[6], which is widely (but not universally) regarded as a result of the ^{234}U being located within α-recoil tracks[7] produced by decay of ^{238}U. The ^{238}U atoms are at the start of the decay chain and are therefore not located in radiation-damaged material.

The preferential leaching in nature of ^{234}U (mean life 3.5×10^5 yr or half-life 2.4×10^5 yr) provides a test of the stability of α-recoil-produced radiation damage over times that are vastly greater than those attainable in the laboratory and in conditions that are relevant to underground storage of nuclear wastes. For minerals where the radiation damage is stable at ambient temperatures, the long-term accelerated ^{234}U leaching will be troublesome for disposal. However, in minerals where chemical attack is not accelerated, we have suitable candidates for waste storage media[8] which could well be useful in other high-radiation environments such as those at fission or fusion reactors.

In contrast to long-lived ^{234}U from primary ^{238}U, the thorium decay product of primary ^{232}Th, ^{228}Th, has a mean life of 2.7 yr. The latter nuclide is formed by decay of the α-recoil atom ^{228}Ra (mean life, 8.3 yr). Therefore, the leaching of ^{228}Th will display the effects of radiation damage that is always relatively fresh. A second radiogenic thorium isotope is available for comparison, namely, the long-lived ^{230}Th (mean life, 1.1×10^5 yr) from the uranium decay chain. We have observed profound differences between the behaviour of the $^{228}Th/^{232}Th$ pair and the pairs $^{230}Th/^{232}Th$ and $^{234}U/^{238}U$.

The stability of a material against radiation damage is closely correlated with its propensity to become amorphous (metamict) during irradiation[9]. Understanding what controls the ease of disordering is a basic question in solid-state physics[10]. For example, Ewing *et al.*[11] have shown how the leachability of zircon increases with the natural internal irradiation dose and Clinard *et al.*[12] have examined ^{238}Pu-doped zirconolite in which damage accumulates rapidly enough for laboratory experiments.

We report here leaching tests on two types of ancient radioactive minerals, thorianite and uraninite, composed primarily of highly-crystalline thorium-rich and uranium-rich ThO_2/UO_2 solid solutions, respectively. Structurally, these are the natural analogues of fluorite-type actinide dioxides, which are important nuclear-waste materials. Included are two thorianite specimens from Sri Lanka (samples T1 and T2) and a uraninite (sample U1) from Wilberforce, Ontario, Canada. Analysis by electron microprobe (EMP) showed that uranium, thorium and lead were uniform within the major phase of each mineral studied (< 10% variation) and that only small-volume fractions of accessory phases were present. The latter was confirmed by the absence of unexplained X-ray diffraction spots. The ThO_2 and UO_2 contents were determined by radiochemical analyses (procedures described in refs 13, 14). Lead isotope analyses with an ion microprobe, together with elemental analyses by the EMP, gave radiometric ages according to the U/Th/Pb methods. These data are given in Table 1, which also lists the number of times the average atom has been displaced by α-recoil and α-particle processes. The ages agree with those previously measured for other samples from the same locale[15]. Note that, if the displaced atoms remained displaced over all of time, the material would be thoroughly and uniformly damaged and therefore would be uniformly leached. But we find that this is not the case.

We subjected the finely pulverized (mean particle diameter of the order of 1 μm, maximum particle size of ~100 μm) mineral samples to a series of successive leaching treatments in a bicarbonate/carbonate solution (0.1 M $NaHCO_3$/0.1 M Na_2CO_3) and then determined the relative leaching rates of ^{238}U, ^{234}U, ^{232}Th, ^{230}Th and ^{228}Th (see refs 13, 14). The enhanced dissolution, shown in Fig. 1, of ^{228}Th relative to its parent ^{232}Th, clearly indicates the presence of localized damage by α-recoil atoms. However, the much smaller isotope fractionation, on dissolution, between ^{234}U and its parent ^{238}U and between ^{230}Th and ^{232}Th suggests that almost complete annealing of the damage occurs in $< 10^5$ yr. Note that the initial reduction in the value of $R(^{228}Th/^{232}Th)$ as dissolution proceeds may be attributed to the rapid depletion of ^{228}Th in the layer of the mineral that is in contact with the solution. However, further exposure of the mineral to the solution causes an increase in the value of $R(^{228}Th/^{232}Th)$. As recently observed for the mineral monazite[13,14], the effect has been caused by retention of ^{228}Ra in the solid phase.

The presence of residual atomic-displacement damage in the minerals is further suggested by two consequences of annealing the samples at 810 °C for ~7 h; the leaching rates are lower and the lattice parameter decreases by 0.2–0.5% (full details are given elsewhere[16]).

The leaching results allow a damage storage-time to be derived. The emerging physical picture is of minerals in which each atom over the age of the mineral has been displaced from its lattice site many times; yet the mineral remains crystalline.

Table 1 Compositions, U/Th/Pb ages and radiation damage in thorianite and uraninite specimens

Mineral	ThO_2 (wt %)*	UO_2 (wt %)*	Age (Myr)†	Dpa‡
Thorianite				
T1	47.6	31.6	550	155
T2	51.9	26.9	550	141
Uraninite				
U1	14.8	61 3	1,000	450

* Uncertainties ~2%.

† Uncertainties ~7% For specimens T1 and T2 a composite age is given.

‡ Estimated displacements per atom, assuming 1,500 atomic displacements per α decay in the uranium, actinium and thorium series.

The inescapable conclusion is that the damage has been healing itself. We now consider the consequences of a steady-state process of damage and recovery, in which severe localized damage from α-recoil events survives for some time before vanishing into a background of more dispersed damage. This mode generates vital annealing-time information. For all three mineral samples, T1, T2 and U1, the value of $R(^{228}Th/^{232}Th)$ was found to be initially much greater than unity (3.8, 6.4 and 2.1, respectively, from Fig. 1), whereas the values of $R(^{234}U/^{238}U)$ were 1.11, 1.12 and 1.08, respectively. As the ^{228}Th is short lived and ^{234}U is long lived, the simplest interpretation of these results is that self-annealing heals the radiation damage in a time between ~11 yr (the combined mean lives of ^{228}Ra and ^{228}Th) and $\sim 3.5 \times 10^5$ yr. Assuming that in the absence of annealing the $^{234}U/^{238}U$ fractionation would have been as high as the observed $^{228}Th/^{232}Th$ fractionation, then the annealing time is τ_{234}, $[R(^{234}U/^{238}U) - 1]/[R(^{228}Th/^{232}Th) - 1]$, where τ_{234} is the mean life of ^{234}U (3.5×10^5 yr) and R is the initial relative isotope leaching rate. (The derivation is given in more detail elsewhere[16].) We find average annealing times[16] of ~15 kyr (14, 7.8 and 25 kyr for the three samples). A time of 18 kyr is implied by the monazite data of Eyal[13,14], who, however, quoted only an upper limit of 10^5 yr.

Two conclusions emerge. First, even highly non-metamict minerals, as usually inferred from X-ray diffraction, may be subjected to radiation damage by α-recoil atoms. The localized submicroscopic variation in atomic order in these minerals may cause preferential leaching and, consequently, radioactive disequilibrium, as is often observed in nature. Second, the method of isotope analysis used here should allow the damage storage time of other minerals to be measured and improved waste-storage materials to be identified.

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1. Burns, W G, Hughes, A E, Marples, J A C, Nelson, R S & Stoneham, A M *Nature* **295**, 130–132 (1982)
2 Matzke, Hj *Rad Effects* **64**, 3–33 (1982)
3 Fleischer, R L *Science* **207**, 979–981 (1980)
4 Fleischer, R L *Geochim. cosmochim Acta* **46**, 2191–2201 (1982)
5 Dran, J C, Maurette, M & Petit, J C *Science* **209**, 1518–1520 (1980)
6 Szabo, B J in *8th INQUA Conf.*, 941–949 (International Union for Quaternary Research, Brussels, 1969)
7 Huang, W H & Walker, R M *Science* **155**, 1103–1106 (1967)
8 Roy, R. & Vance, E R *J Mater Sci* **16**, 1187–1190 (1981).
9 Mitchell, R S *Miner Rec* **4**, 177–182 (pt 1), 214–223 (pt 2) (1973)
10 Naguib, H M. & Kelley, R *Rad Effects* **25**, 1–12 (1975)
11 Ewing, R. D, Haaker, R F & Lutze, W in *Scientific Basis for Radioactive Waste Management* V (ed Lutze, W.) 389–397 (Elsevier, Amsterdam, 1982)
12 Clinard, F W. *et al. J nucl. Matter* **105**, 248–256 (1982).
13 Eyal, Y & Kaufman, A *Nucl. Tech* **58**, 77–83 (1982)
14 Eyal, Y in *Scientific Basis for Radioactive Waste Management* V (ed Lutze, W) 399–408 (Elsevier, Amsterdam, 1982)
15 Faul, H *Nucl Geol* (Wiley, New York, 1954)
16 Eyal, Y & Fleischer, R L *Geochim cosmochim. Acta* **49**, 5 (1985)

A thermo-mechanical model of continental lithosphere

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An understanding of the mechanical response of the continental lithosphere to vertical surface loading is crucial when considering geodynamic processes such as intraplate basin formation, post-glacial rebound or mountain building. Previous models of the mechanical behaviour of the lithosphere, which are more successful in oceanic than in continental regions, have used specific isotherms to delineate an effectively elastic zone of the lithosphere and result in mathematically convenient descriptions of mechanical behaviour. Heat flow and lithosphere flexure data for continents, however, suggest that this relationship is more complex, which can be demonstrated by numerical modelling of the loading of a heterogeneous visco-elastic lithosphere with temperature-dependent viscosity. Viscous stress relaxation in the lower and hotter section of the lithosphere results in a region of stress concentration dependent on the thermal state of the lithosphere, its structure and the time since loading. Here we present initial numerical models, based on a relatively simple steady-state thermal model constrained by present-day heat flow, which explain observations of continental flexure except in regions which have experienced a long or complex thermal history since loading.

As a first approximation, both oceanic and continental lithosphere loading have been modelled as a flexed elastic plate overlying an inviscid fluid substratum[1-3]. But laboratory studies of the deformation of rocks[4-6] and detailed modelling of the above phenomena[7-10] suggest the actual rheology of the lithosphere must be considerably more complex. Based on studies of the deformation of olivine, Goetze and Evans[7] proposed a rheology in which the uppermost lithosphere is weakened by brittle failure and stresses in the lower lithosphere are relaxed by a temperature-dependent non-linear dislocation flow mechanism, leaving most recoverable strain in the region between. They further suggested that the temperature-dependent flow could be approximated by a plastic failure criterion. This allows a time-independent calculation of stress distribution and, more specifically, predicts an observable property of apparent elastic lithosphere thickness. This yield-stress envelope model was applied with particular success to oceanic lithosphere flexure[9,11,12] and assisted in explaining the apparent correlation between elastic thickness and depth to the 450 °C isotherm as calculated from the cooling plate model associated with sea-floor spreading[2,13].

Recently, Karner *et al.*[14] applied this thermo-elastic model to continental lithosphere flexure. Their analysis of flexural rigidity of continental lithosphere in terms of thermal age of the lithosphere at the time of loading led them to conclude the following: (1) flexural rigidities for continental lithosphere are compatible with oceanic results and are explained universally by a simple thermo-elastic model; (2) the long-term thermal behaviour of continental lithosphere is governed by a simple cooling plate model with a 200–250-km plate thickness; (3) the effective elastic thickness of continental lithosphere coincides with the depth to the 450° isotherm at the time of loading and does not change with time. Although we find support in heat-flow data for the basic contention of Karner *et al.*[14] that lithosphere mechanical behaviour is controlled largely by its thermal state, the precise relationship between thermal state and mechanical behaviour may be more complex than their conclusions suggest and we offer here an alternative explanation for continental flexure observations.

Table 1 is a compilation of continental flexure observations and estimates of present surface heat flow at each site. The latter values are generally averages of many heat-flow determinations with effects such as anomalous radiogenic heat production of surface rocks, or possible hydrological effects weighted so that the average heat flow is a reliable indicator of lithospheric thermal state. We make two observations from these data. First, the present thermal state of continental lithosphere at flexure sites is not a simple function of its age (compare column 6 with columns 3 and 4 in Table 1). This observation is consistent with results obtained from much larger heat-flow data sets not restricted to flexure sites[15-17] and should be considered in judging the general applicability of a thermal evolution model. Second, apparent elastic thicknesses for continental flexure sites do not correspond to the 450 °C-isotherm position. In fact, by using present-day heat flow to compute the present thermal state, we see that elastic thicknesses correspond to depths for a variety of isotherms from 300 °C to >900 °C (Fig. 1). The thermal regime has not necessarily been constant since loading. But as a plastic yield-stress model responds to the warmest thermal state following loading, the data (Fig. 1) represent lower limits along the surface heat-flow axis. The observation of very high temperatures or of a wide range of temperatures controlling elastic thickness is inconsistent with the oceanic thermo-mechanical model.

Is there a rational explanation for the apparent success of the plastic yield-stress approximation in explaining oceanic lithosphere flexure but its lack of success for the continents? Courtney and Beaumont[18] have demonstrated that the rapid cooling of oceanic plates effectively locks in the stress field and prohibits significant stress relaxation. In continental lithosphere subjected to a longer and more complex thermal history, we believe this total damping of the long-term relaxation may not be taking place. However, this seeming contradiction can be examined by obtaining complete time-dependent solutions to the more general visco-elastic problem.

To obtain a time-dependent solution of lithosphere flexure with a non-linear thermally activated rheology, we must resort to numerical methods. The model we have developed consists of a heterogeneous Maxwell visco-elastic lithosphere overlying an inviscid asthenosphere. The viscous strain rates $\dot{\varepsilon}$ in the lithosphere are determined by a power law ($n=3$ in stress σ) which is exponentially dependent on absolute temperature T through an activation energy E^*, that is, $\dot{\varepsilon} = A\sigma^n \exp(-E^*/RT)$, where A, n and E^* are properties of the material and R is the gas constant. For the mantle we have used the experimentally determined flow parameter for olivine given by Goetze[5] ($A=7\times10^4$ MPa^{-3} s^{-1}, $E^*=0.53$ MJ mol^{-1}). Parameters for crustal rocks are difficult to determine because of the scarcity of data, but seem to have consistently lower activation energies. We used values of $A=7\times10^{-6}$ MPa^{-3} s^{-1} and $E^*=0.23$ MJ mol^{-1}. The thickness of the lithosphere is determined by its thermal state; its base is taken to be approximately the position where the geotherm intersects the 1,300 °C adiabat. A vertical uniformly-distributed surface load of width equal to the lithosphere thickness and infinite length is applied to the upper surface. This load is supported by buoyancy forces resulting from flow in the mechanical asthenosphere. The mechanical strength of the lithosphere determines the redistribution of these opposing forces. We have used a finite element method to solve for the time-dependent displacements and stresses within the visco-elastic body incorporating an algorithm and code from Owen and Hinton[19]. (Details of this modelling are given in Willett *et al.*[20].)

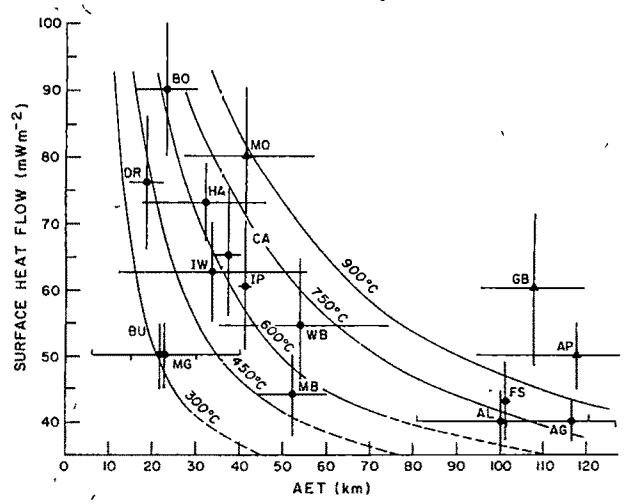


Fig. 1 Surface heat flow at selected flexure sites versus AET. Data and symbols from Table 1. Solid lines indicate depths to isotherms at a given heat flow for a steady-state thermal model.

Salient features of the flexure of a continental lithosphere with this rheology are shown in Fig. 2*a*, *b*. These two examples are chosen to represent end-member thermal states for continental lithosphere. Fig. 2*a*, labelled 'cold lithosphere', represents deformation of a lithosphere with a vertical temperature distribution corresponding to a steady-state geotherm with surface heat flow of 40 mW m^{-2}; Fig. 2*b*, labelled 'hot lithosphere', has a 90 mW m^{-2} or active-rift geotherm[21]. The displacements in both cold and hot lithosphere show characteristics of visco-

Table 1 Continental lithosphere age, flexure and thermal characteristics*

Site	Symbol	Basement age at time of loading (Myr BP)	Age of load (Myr BP)	AET (km)	Present heat flow (mW m^{-2})	Heat-flow reference
Lake Agassis	AG	1,600–1,800	0.007–0.014	101–132	40±3	24
Lake Algonquin	AL	1,200–1,500	0.0011–0.0013	80–120	40±5	24
Fennoscandia	FS	2,000–2,800	0.012	101	43±7	25
Caribou Mts	CA	1,600–1,800	1–10	34–40†	65±10	26
Interior Plains	IP	1,600–2,500	1–10	41†	60±10	27
Ganges Basin	GB	1,000–2,000	10–50	95–120	60±12	28, 29
Idaho–Wyoming Thrust	IW	75–275	50–150	12–55	62±8	30
Michigan Basin	MB	800–1,100	370–460	44–60	44±6	30
Boothia Uplift	BU	1,000–1,400	460–550	6–40†	50±5	27
Midcontinent Gravity High	MG	100–400	1,100	15–30	50±5	30
Lake Hamilton	HA	200	0.009–0.019	18–46	73±6	31
Lake Bonneville	BO	200	0.015–0.020	16†	90±10	32
North Great Dividing Range	DR	0–100	400–500	15–22	76±10	33
Appalachian Foreland Basin	AP	560–1,000	330–390	95–140	50±5	30
Molasse Basin	MO	230–320	20–30	26–56	80±10	34
Williston Basin	WB	2,000–2,400	110–450	34–75‡	55±10	35

* Modified from ref. 23; † data from ref. 1; ‡ data from ref. 36.

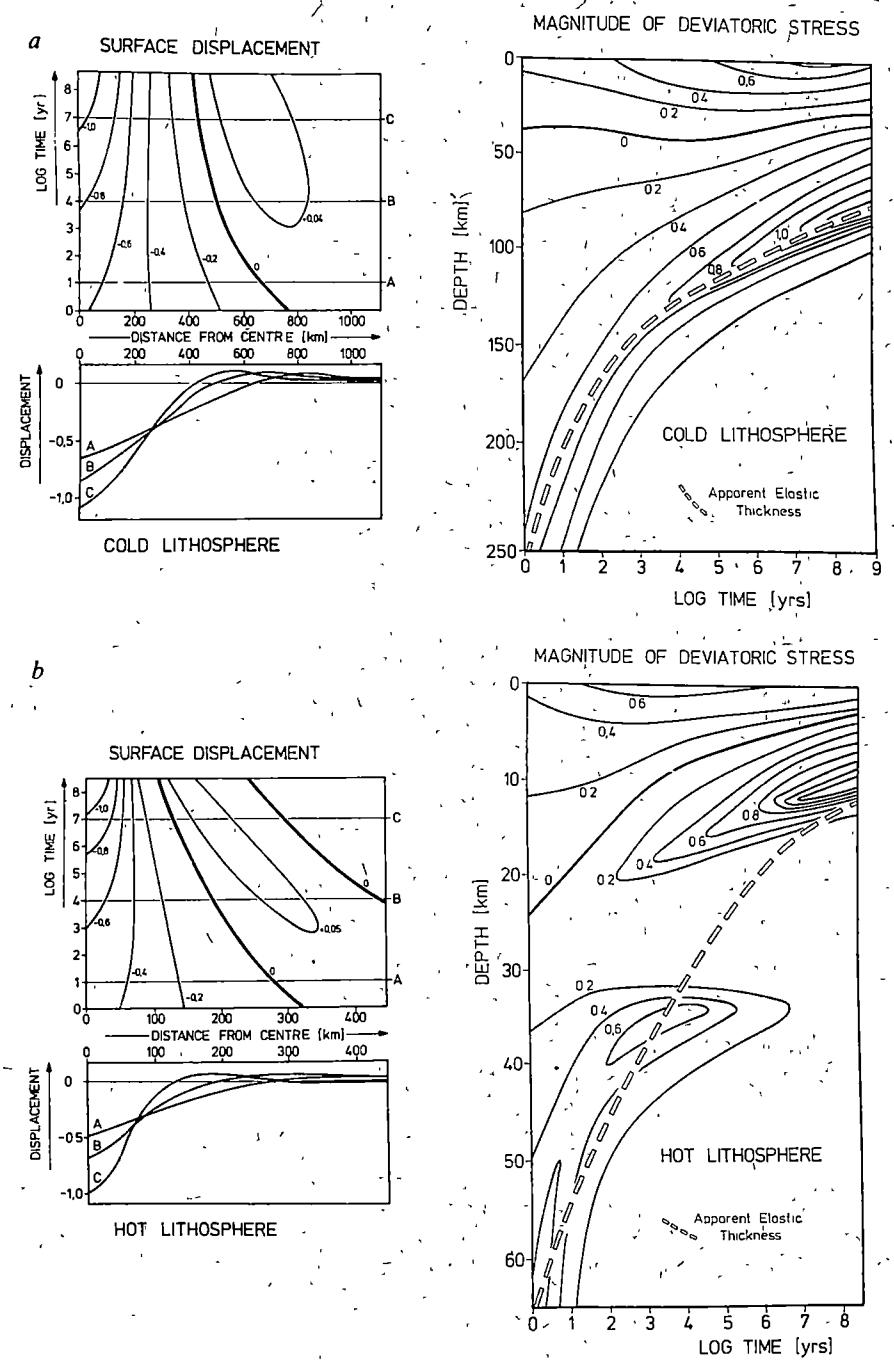


Fig. 2 Flexure properties for two end-member thermal states of visco-elastic continental lithospheres. In *a* and *b*, the upper left figure shows surface vertical displacements mapped as a continuous function of time; lower left figure gives the original surface (that is, basement) profile at three distinct times. Right figures show deviatoric stresses on a plane directly below the centre of the load and the temporal evolution of this stress field following instantaneous loading at time zero. Dashed line gives apparent elastic thickness as calculated from the wavelength of the surface displacements. The 'cold lithosphere' has a vertical temperature distribution corresponding to a geotherm with a surface heat flow of 40 mW m^{-2}; displacements are normalized to 1,200 m; stresses are normalized to 50 MPa. The 'hot lithosphere' has a temperature distribution corresponding to a geotherm with a surface heat flow of 90 mW m^{-2}; displacements are normalized to 1,600 m; stresses are normalized to 100 MPa.

elastic plate flexure: a central depression, deepest at the centre, which deepens and narrows with increasing time; and the development of a region of vertical uplift, a peripheral bulge, which in time migrates towards the basin.

The stress field also shows the basic elements of flexural response. At any given time it includes a plane of zero deviatoric stress separating regimes of horizontal extension and compression. The magnitude of the stress reaches a maximum at the surface or base of the mechanical lithosphere. Immediately after the onset of loading, stresses at the base of the lithosphere begin to relax and a region of effectively zero deviatoric stress develops. As time progresses this relaxed region grows upward, thereby concentrating stress in the upper lithosphere, a phenomenon which has also been observed in other geodynamic models[18,22]. The region of negligible deviatoric stress acts as the mechanical asthenosphere. Because of the strong temperature dependence of flow rates, the rate of upward concentration of stresses decreases with time, allowing the colder upper lithosphere to maintain stresses. This relaxation pattern is changed with an increase in heat flow and corresponding increase in temperature as seen in the 'hot lithosphere' model. The increase in temperature activates flow in the lower crust and, consequently, stresses are relaxed quickly in the lower crust as well as in the lower lithosphere. This second zone of relaxation forces the upper 15–20 km to carry the entire load. The rheology of the crust controls lithosphere response in high temperature regimes.

The flexural wavelength of the surface displacements is used to calculate an apparent elastic thickness (AET) at each point in time. This AET has been superimposed on the stress fields of Fig. 2 and approximately corresponds to the maximum depth to which deviatoric stresses are maintained. Given the logarithmic scales in Fig. 2, it seems that little change in the stress and displacement fields takes place at longer times, an observation which supports the validity of an elastic–plastic model approximation. However, the long term value of elastic thickness is not a simple function of temperature and, until the processes determining this value are understood, we retain the complete solution.

For use in predicting flexural parameters, our model results are summarized in Fig. 3*a*. Each contour gives the apparent

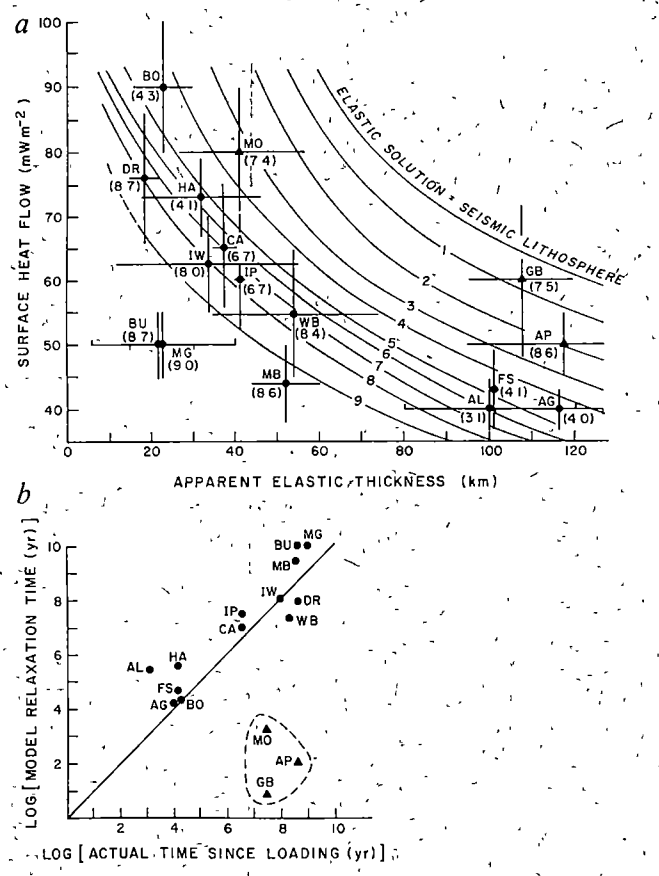


Fig. 3 *a*, Relaxation map relating apparent elastic thickness of a visco-elastic continental lithosphere to thermal state and loading time. Data and symbols from Table 1. Numbers in parentheses represent $\log_{10}$ (actual load duration in yr) for each site. Solid contour lines show the predicted weakening of the lithosphere with time after loading, caused by viscous stress relaxation in the lower and hotter part of the lithosphere; contour values are $\log_{10}$ (load duration in yr). Irregular contours result from lithosphere heterogeneity that affects the entire stress-relaxation process, and from numerical model interpolation. *b*, A comparison of the actual time since loading versus the visco-elastic model relaxation time necessary to produce the observed apparent elastic thickness. Model data are interpolated from relaxation map in *a*. Triangles are foreland basin sites in collision zones.

elastic thickness, as calculated from the visco-elastic model, for a given thermal state and a particular loading time. For example, a hot lithosphere characterized by surface heat flow of 90 mW m^{-2} (for example, Basin and Range) has an AET of 65 km for very short loading times (seismic lithosphere), which relaxes to <30 km in 10^4 yr (Lake Bonneville unloading) and would further relax to an AET of 15 km were the load and the same thermal state to exist for 10^8 yr. In contrast, an intermediate 'warm' lithosphere characterized by surface heat flow of 60 mW m^{-2} starts out with a seismic lithosphere of 125 km, relaxes to an AET of 50 km in 10^6 yr after loading and to an AET of 38 km after 10^8 yr. We have also superimposed on this relaxation map (Fig. 3*a*) the flexure data from Fig. 1. Included with each data point is the age (duration) of the load. There is general agreement between actual load duration and model relaxation time necessary to reach the observed AET at a given thermal state, as shown by the near one-to-one correspondence in Fig. 3*b*, despite large uncertainties in both thermal and flexure data and the sensitivity of the relaxation map to model parameters. The fit in Fig. 3 is surprisingly good considering that the relaxation contours in Fig. 3*a* were calculated from models with general loading conditions and flow properties and a simple thermal model. Furthermore, as this relaxation model was constructed from steady-state thermal models, we would expect to fit only those data for which the thermal state has not changed appreciably since loading. For those data with long or complex thermal histories large errors in model fit could exist.

This is probably the case with the foreland basin data (MO, AP, GB), which depart dramatically from model predictions and misfit the model in Fig. 3. The lithosphere flexure in continental collision zones is the result of complex thermal and mechanical processes which apparently are not simulated by this model. It is less likely, but also possible, that heat-flow determinations surrounding foreland basins are systematically biased. The oldest loads (BU, MG, MB) have had a greater opportunity to experience transient thermal effects and so are more likely not to fit this steady-state model. This may be the case, but is obscured by the logarithmic scales in Fig. 3.

Our analysis of continental flexure data together with heat-flow data produces the following conclusions: (1) The application of oceanic lithosphere thermal and thermo-mechanical models to continental lithosphere does not seem to be supported by heat-flow and continental flexure data. In particular the oceanic lithosphere correlations between heat flow and age and between elastic thickness and a specific temperature range are not observed on continents. (2) Complete time-dependent solutions for visco-elastic lithospheric flexure with thermally activated flow properties can be obtained numerically, the principal free parameters being temperature and rheological properties. Generic visco-elastic mechanical models of a vertically heterogeneous lithosphere incorporating simple steady-state thermal models can explain most observed flexural parameters. Those data that are not well fit by the model have long or complex thermal or mechanical histories. An understanding of both is critical when considering the long-term behaviour of continental lithosphere.

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1 Walcott, R. I *J geophys Res* **75**, 3941-3954 (1970)
2 Caldwell, J G. & Turcotte, D L *J geophys Res* **84**, 7572-7576 (1979)
3 Karner, G. D & Watts, A B *J geophys Res* **88**, 10449-10477 (1983)
4 Ashby, M F & Verrall, R. A *Phil. Trans. R. Soc* **A288**, 59-95 (1977)
5. Goetze, C *Phil Trans R. Soc.* **A288**, 99-119 (1978)
6 Kirby, S. *Rev Geophys. Space Phys* 1458-1487 (1983).
7. Goetze, C. & Evans, B. *Geophys J. R. astr Soc* **59**, 463-478 (1979)
8 Watts, A B, Bodine, J H. & Steckler, M S *J geophys Res* **85**, 6369-6376 (1980)
9 McNutt, M K & Menard, H. W *Geophys J R astr Soc* **71**, 363-394 (1982)
10 Quinlan, Garry M & Beaumont, C *Can J. Earth Sci* (in the press)
11 Watts, A B & Talwani, M. *Geophys J. R. astr. Soc* **36**, 57-90 (1974)
12. Bodine, J H, Steckler, M S & Watts, A B *J geophys Res* **86**, 3695-3707 (1981)
13 Parsons, B & Sclater, J G *J geophys Res* **82**, 803-827 (1977).
14 Karner, G. D, Steckler M S & Thorne, J. A *Nature* **304**, 250-253 (1983)
15 Rao, R. U M., Rao, G V & Reddy, G K *Earth planet Sci. Lett* **59**, 288-302 (1983)
16 Chapman, D S, Germann, D. & Neugebauer, H J *Terra Cognita* **3**, 95 (abstract) (1983)
17 Morgan, P. & Sass, J H *J Geodynamics* **1**, 143-166 984)
18 Courtney, R C & Beaumont, C *Nature* **305**, 201-204 983).
19 Owen, D. R J & Hinton, E *Finite Elements in Plasticit,* (Pineridge, Swansea, 1980)
20 Willett, S D, Chapman, D S & Neugebauer, H J *Ann Geophys* (in the press)
21 Pollack, H N & Chapman, D S *Tectonophysics* **38**, 279-296 (1977)
22 Kusznir, N S & Park, R G. *Nature* **299**, 540-542 (1982)
23 Watts, A B, Karner, G D & Steckler, M S. *Phil Trans R Soc.* **A305**, 249-281 (1982)
24 Jessop, A M. & Lewis, T J *Tectonophysics* **50**, 55-77 (1978)
25 Cermak, V in *Terrestrial Heat Flow in Europe* (eds Cermak, V & Rybach, L) 3-40 (Springer, Heidelberg 1979)
26 Majorowicz, J A & Jessop, A M *Tectonophysics* **74**, 209-238 (1981)
27. Jessop, A M, Hobart, M A & Sclater, J G *Geotherm Ser* **5**, (Geothermal Service of Canada, Earth Physics Branch, Ottawa, 1976)
28 Rao, R. U M., Rao, G. V. & Narain, H *Earth planet Sci Lett* **30**, 57-64 (1976)
29 Rao, G V & Rao, R U M *Earth planet Sci Lett* **48**, 397-405 (1980)
30 Sass, J H *et al* in *McGraw-Hill/CINDAS Data Series on Material Properties* Vol. II-2 (eds Touloukian, Y S, Judd, W R & Roy, R F 503-548 (McGraw-Hill, New York 1981)
31 Lewis, T. J & Jessop, A M *Can J Earth Sci* **18**, 366-375 (1981)
32. Chapman, D S *Abstracts of papers presented at Interdisciplinary Symposia* **14**, 436 (XVII General Assembly of the IUGG, Canberra, Australia, 1979)
33 Cull, J P & Denham, D *Bur Miner Resources J Aust Geol Geophys* 4(1), 1-13 (1979)
34 Haenel, R in *Atlas of Subsurface Temperatures in the European Community* (eds Haenel, R *et al*) (Th Schaefer, Hannover, 1980)
35 Gosnold, W D Jr *Bulletin* No. 84-04-MMRRI-04, 1-110 (Mining and Mineral Resources Research Institute, University of North Dakota, Grand Forks, 1984)
36 Ahern, J L & Mrkvicka, S R. *Tectonics* **3**, 79-102 (1984)

Lateral transport of Mn in the north-east Pacific Gyre oxygen minimum

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Accurate measurement of the concentration and fluxes of a wide range of dissolved and particulate materials in the water column is need to quantify the global cycling of heat, water and chemical constituents[1]. An important aspect of any Global Ocean Flux study is the lateral transport of materials at basin or global scales; to study lateral transport, useful tracers of water-mass movements must be identified. Here we report that Mn is rapidly removed (-0.027 μmol m^{-3} yr^{-1}) from the north-east Pacific Gyre dissolved-Mn maximum onto biogenic particles sinking through the oxygen minimum (500–1,100 m). To support removal, dissolved Mn must be supplied within the oxygen minimum at net advective horizontal transport rates of ~0.6–0.9 cm s^{-1}. The high affinity of dissolved Mn for particles, together with the readily discernible differences in inshore/offshore dissolved distributions, indicate that Mn is a chemical tracer with great potential in future studies of large-scale circulation processes in the ocean[1].

We show dissolved and suspended particulate Mn distributions observed 830 km north of the Hawaiian Islands (28°N; 155°W), together with temperature, salinity and dissolved oxygen data, in Fig. 1. Dissolved-Mn concentrations were relatively high (0.8 nmol kg^{-1}) in near surface waters, then decreased steadily until a minimum (~0.2 nmol kg^{-1}) was reached at depths of 200–450 m (probably originally subarctic surface waters). Dissolved Mn began increasing concomitantly with rapidly decreasing oxygen concentrations in the 450–700-m depth interval. After reaching a maximum of 0.54 nmol kg^{-1} at 700 m, concentrations decreased to 0.34 nmol kg^{-1} at 1,000 m. With the exception of two relatively high values observed at 1,090 m (0.55 nmol kg^{-1}) and 1,420 m (0.40 nmol kg^{-1}), amounts of dissolved Mn remained fairly constant (0.24–0.29 nmol kg^{-1}) for the remainder of the 2,090-m water column sampled. These data were obtained using GoFlo-bottle sampling, Chelex-100 ion-exchange preconcentration and atomic absorption analytical procedures that are described in detail elsewhere[2].

In addition to the dissolved amounts, two fractions of suspended particulate Mn were also measured: weakly leachable (LE, that dissolved with 25% acetic acid)[3]; and that remaining after leaching (RE, the refractory portion, dissolved via Teflon bomb digestion)[4]. The latter is believed to be that locked into aluminosilicate lattices and, as expected, amounts were extremely low (0.2–5.9 pmol kg^{-1}) in these remote gyre waters far removed from continental land masses (Fig. 1). Particulate LE Mn concentrations were very low (4–9 pmol kg^{-1}) in the upper 140 m of the water column (Fig. 1). More or less steadily increasing values were found from 140–600 m, followed by a minimum at 900 m, a secondary maximum at 1,090 m and steadily decreasing concentrations thereafter.

Because free-floating particle traps[5] were also used during this study (deployed for 33 days), flux data are available to determine whether Mn was being regenerated from (decreasing fluxes with depth), or scavenged onto (increasing fluxes with depth), particles sinking through the oxygen minimum. The dramatic increases in Mn fluxes (Fig. 2) indicate, in view of the following considerations, that dissolved Mn was being removed onto particles. Increases in total flux did not result from the lateral transport of aluminosilicate phases because RE Mn fluxes remained uniformly low and constant (2–3 μmol m^{-2} yr^{-1}) throughout the 2,000-m water column (Fig. 2). Furthermore, the RE Mn fluxes were similar to atmospheric air/sea Mn fluxes (3.3 μmol m^{-2} yr^{-1}) estimated by Duce at Eniwetok[6], and we assume that this was the primary source of the aluminosilicate (that is, RE) Mn in our traps.

Trapped exchangeable (TE, that dissolved in the particle trap cyclinders)[2,7,8] Mn fluxes were also fairly constant with depth (14–20 μmol m^{-2} yr^{-1}), though they were an order of magnitude greater than the RE Mn fluxes. Clearly then, the fraction responsible for the dramatic increase in total Mn fluxes between 500 and 1,000 m was the LE fraction (Fig. 2). This flux increase could result from *in situ* scavenging of dissolved Mn or from the advective lateral transport of particles already rich in LE Mn. Comparisons of suspended particulate LE Mn and trapped particulate LE Mn concentrations (Fig. 1) indicate that the trapped particles are remarkably different from those in suspension (at least judging by their Mn content) for most of the water column; that is, Mn concentrations are not equivalent in the two classes of particles until depths of 900 m are reached. Thus, we conclude that the increase in flux does indeed result from the *in situ* removal of dissolved Mn onto biogenic particles sinking through the O_2 minimum/Mn maximum.

Assuming steady-state conditions, this removal of dissolved Mn must be balanced by input processes of some kind. To define the importance of various input mechanisms, we used a simple 500–1,100-m box model shown in Fig. 3. The model illustrates the following: (1) Mn removed from the box by particle scavenging at a rate of -0.027 μmol m^{-3} yr^{-1} (500-m(LE+TE)Mn flux minus the 1,100-m(LE+TE) Mn flux divided by 1,100 − 500 m = 600 m) would be completely removed in 16 yr because the average integrated Mn concentration in the box is only

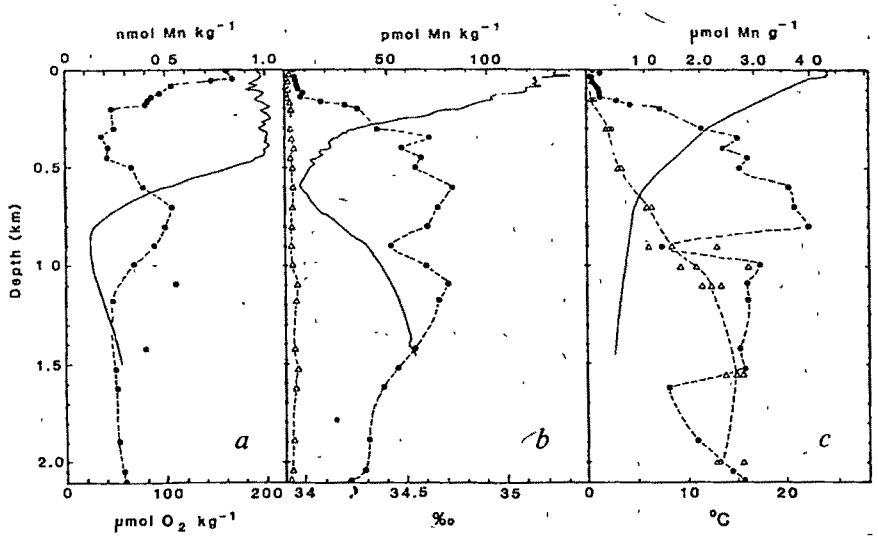


Fig. 1 Manganese and hydrographic measurements made 830 km north of Hawaii (28°N; 155°W) in July/August of 1983: *a*, dissolved Mn (-●--) and oxygen (—); *b*, suspended particulate leachable (-●--) and refractory (-△--) Mn with salinity (——); *c*, suspended (-●--) and trapped (-△--) particulate Mn concentrations on a gram-dry-weight basis, together with temperature (——) data.

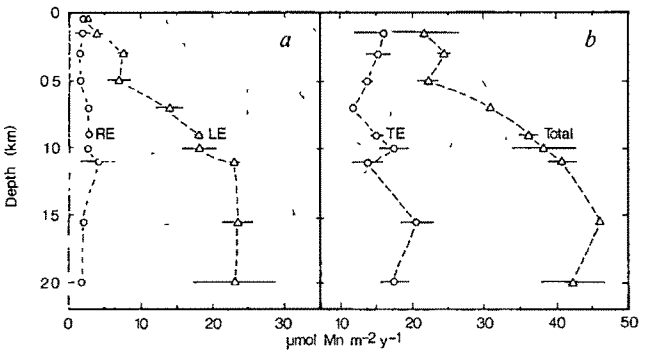


Fig. 2 Manganese fluxes measured with particle traps at 28°N, 155°W *a*, particulate refractory (RE) Mn (-○- -) and particulate leachable (LE) Mn (-△- -); *b*, trapped exchangeable (TE, Mn dissolved in trap solutions) (-○- -) and total Mn fluxes (-△- -). Means and standard deviations ($n=3$) are shown for all depths except 700 m (LE, $n=2$, range is shown, TE and total, $n=1$). Standard deviations for the refractory portions were usually smaller than the symbols used; 50-m TE and total fluxes are not shown because of possible zooplankton swimmer[5] contamination.

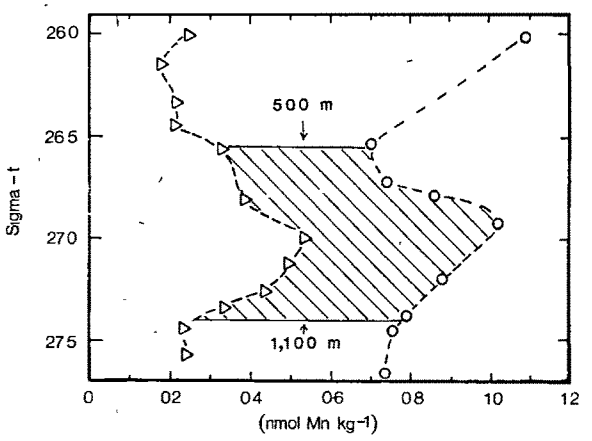


Fig. 4 Dissolved Mn versus density (σT) north of Hawaii (Δ 28°N, 155°W) and west of California (○ 36.5°N, 123°W). Hatched area represents Fig. 3 box model based on Hawaii depth-versus-σT relationship.

0.42 μmol m^{-3}. (2) The shape of the dissolved-Mn profile (Figs 1, 3) shows that vertical advective/diffusive processes cannot be re-supplying the Mn removed by scavenging. Dissolved Mn advecting upward into the box at 1,100 m is balanced by Mn advected upward out of the box at 500 m because the concentrations at 500 and 1,100 m are about the same and there is no reason to believe that vertical advection rates would be markedly different at these depths (for example, we use a velocity of 4 m yr^{-1} at both depths)[9]. (3) It is also apparent that diffusive mixing processes are removing Mn from, rather than supplying Mn to, the box; $\Delta Mn/\Delta z$ calculations from 400–600 m and 1,000–1,200 m result in an upward Mn flux of 5 μmol m^{-2} yr^{-1} across the 500-m top of the box and a downward flux of 4 μmol m^{-2} yr^{-1} across the 1,100-m bottom of the box when a global ocean average eddy diffusivity constant of 1.7 cm^2 s^{-1} is used[10]. (4) Adding up the total vertical fluxes into (22 μmol m^{-2} yr^{-1}) and out of (−47 μmol m^{-2} yr^{-1}) the box results in a difference of 25 μmol m^{-2} yr^{-1} that must be supplied by horizontal advective/diffusive processes to maintain steady-state conditions.

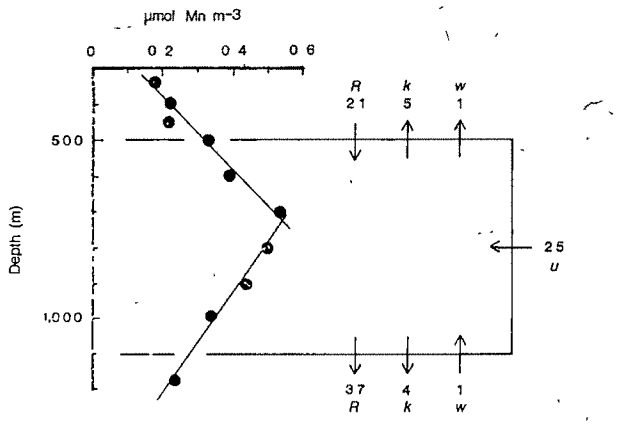


Fig. 3 A box model for the 500–1,100-m depth interval with fluxes (arrows) in units of μmol Mn m^{-2} yr^{-1}. Dissolved Mn versus depth regressions shown on the left side yielded equations of μmol Mn m^{-3}. $= -0.18 + 0.0010$ (m) for the upper line ($r = 0.9758$; $n = 6$) and $= 1.05 - 0.0007$ (m) for the lower line ($r = 0.9890$; $n = 5$) Vertical diffusive fluxes (k) were estimated using these slopes and an eddy diffusivity coefficient of 5,400 m^2 yr^{-1} (1 7 cm^2 s^{-1}). Vertical advective fluxes (w) across 500 and 1,100-m boundaries were estimated by multiplying Mn concentrations by a vertical advective rate of 4 m yr^{-1} Particulate associated (LE + TE) Mn flux data (R) are from Fig 2. The lateral flux (u) was estimated from the difference (total fluxes out of box minus total fluxes into box).

We believe that the source of the Mn being removed in the north-east Pacific Gyre is provided by Mn regeneration[2,7,11,12] along continental margins. For example, dissolved-Mn profiles measured off California[13] are remarkably similar in shape to those measured in this study, when they are compared with sigma-t (σT) data (see Fig. 4). Concentrations are also significantly higher inshore, which must be so if onshore/offshore lateral transport arguments are to be used.

We have no evidence that the Mn in the gyre indeed originated off the west coast of the United States. However, assuming that inshore Mn concentrations along other north Pacific continental margins are about the same as those we show in Fig. 4 and that the distance from California Station to the gyre station is representative (3,150 km), we can make first-order transport estimates assuming that $\Delta Mn/\Delta t = (\Delta Mn/\Delta x)u$, where $\Delta Mn/\Delta t$ is the combined average removal of dissolved Mn from the 500–1,100-m box by particulate scavenging and vertical mixing processes (−25 μmol m^{-2} yr^{-1} − 600 m = −0.042 μmol m^{-3} yr^{-1}); $\Delta Mn/\Delta x$ is the average difference between inshore and offshore dissolved-Mn concentrations in the oxygen minimum (0.46 μmol m^{-3}, from Fig. 4) divided by the distance (3.15×10^6 m) between the two locations; and u is the resulting net advective velocity in units of m yr^{-1}.

Using these estimates, the net advective velocity necessary to support Mn removal within the oxygen minimum off Hawaii is 290,000 m yr^{-1} or 0.9 cm s^{-1}. Based on particulate scavenging rates alone (ignoring vertical mixing processes), the estimates become 180,000 m yr^{-1} or 0.6 cm s^{-1}. Thus, the average length of time required to transport the dissolved Mn from California to Hawaii would be ~10–20 yr.

Clearly, the net advective transport rates presented above are crude first estimates. We considered Mn transport only along the x and z axes, but dissolved Mn may be gained or lost by lateral mixing processes along the y axis as well. Nevertheless, with appropriate expansion of the water column and particle-trap sampling grid, and the simultaneous measurements of other trace elements that are removed in the open ocean (for example, J.H.M. and G.A.K., in preparation) or along continental margins (for example, Ag, Cu)[14] as well as radionuclides (^{210}Pb, ^{231}Pa, ^{230}Th)[15,16], we believe that the use of these chemical tracers will result in a significant gain in the understanding of global ocean circulation processes.

Finally, we believe that the primary processes responsible for the excess Mn accumulating on the sea floor[17,18] are the combination of continental weathering input, dissolved-Mn lateral transport and sinking biogenic particle scavenging output. Although

we have presented these arguments before in descriptions of research performed at pronounced oxygen minimum[2,8] and/or nearer the shore[7,19], final proof required the strong evidence provided here of at depth lateral transport and significant particulate scavenging of dissolved Mn at an open ocean location.

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1 Steele, J H *EOS* **65**, 684 (1984)
2 Martin, J H & Knauer, G A *Earth planet Sci Lett* **67**, 35-47 (1984)
3 Chester, R & Hughes, M J *Chem Geol* **2**, 249-262 (1967)
4 Eggimann, D W & Betzer, P R *Analyt Chem* **48**, 866-890 (1976)
5 Knauer, G A, Martin, J H & Bruland, K W *Deep-Sea Res* **26**, 97-108 (1979)
6 Buat-Menard, P in *Air Sea Exchange of Gases & Particles* (eds Liss, P S & Slinn, W G N) 455-532 (Reidel, Holland, 1983)
7 Martin, J H & Knauer, G A *Deep-Sea Res* **30**, 411-423 (1983).
8 Martin, J H & Knauer, G A *J mar Res* **40**, 1213-1225 (1982)
9 Broecker, W S *Chemical Oceanography* (Harcourt Brace Jovanovich, New York, 1974)
10 Li, Y-H, Peng, T H, Broecker, W S & Ostlund, H G *EOS* **63**, 971-972 (1982)
11 Trefry, J H & Presley, B J *Geochim cosmochim Acta* **46**, 1715-1726 (1982)
12 Kremling, K *Nature* **303**, 225-227 (1983)
13 Knauer, G A, Martin, J H & Gordon, R M *Nature* **297**, 49-51 (1982)
14 Martin, J H, Knauer, G A & Gordon, R M *Nature* **305**, 306-309 (1983)
15 Bacon, M P, Spencer, D W & Brewer, P G *Earth planet Sci Lett* **32**, 277-296 (1976)
16 Anderson, R F, Bacon, M P & Brewer, P G *Earth planet Sci Lett* **66**, 73-90 (1983)
17 Bender, M L, Ku, T-L & Broecker, W S *Earth planet Sci Lett* **8**, 143-148 (1970).
18 Bender, M L, Klinkhammer, G P & Spencer, D W *Deep-Sea Res* **24**, 799-812 (1977)
19 Martin, J H & Knauer, G A *Earth planet Sci Lett* **51**, 266-274 (1980)

Sr isotope composition of sea water over the past 75 Myr

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The $^{87}Sr/^{86}Sr$ ratio of ancient seawater, as recorded in marine carbonates, is an important tracer of long-term variations in ocean chemistry[1-5]. However, the Sr isotope balance of the oceans has been difficult to constrain; consequently, attempts to evaluate the temporal $^{87}Sr/^{86}Sr$ changes have been largely qualitative. To constrain the causes of these variations we have measured $^{87}Sr/^{86}Sr$ ratios in carefully cleaned unrecrystallized foraminifera from DSDP sites 21 and 357. The data presented here have been quantitatively modelled taking advantage of recent advances in understanding of the Sr geochemical cycle. They suggest that whereas hydrothermal fluxes and carbonate recycling are of major importance in defining the marine $^{87}Sr/^{86}Sr$ ratio, the major control over its variations through the Cenozoic has been changes in the isotope composition of Sr derived from the weathering of silicate rocks.

Previous studies have suggested that the major controls over variations in the marine $^{87}Sr/^{86}Sr$ ratio are: (1) the proportions of oceanic Sr derived from crustal (mainly granitic) and mantle (continental basalts and hydrothermal input) sources; (2) the rate of transport of Sr from the continents to the oceans; and (3) recycling of Sr from marine carbonate sediments[2,5-9]. Two factors require that these ideas and the data behind them be re-examined. First, an increased understanding of the magnitude of the hydrothermal processes at ocean ridges allows greater constraints to be placed on the oceanic Sr isotope balance[10-13]. Second, a recently-produced Sr isotope curve for seawater shows more fluctuations through time than were previously thought to be present[1].

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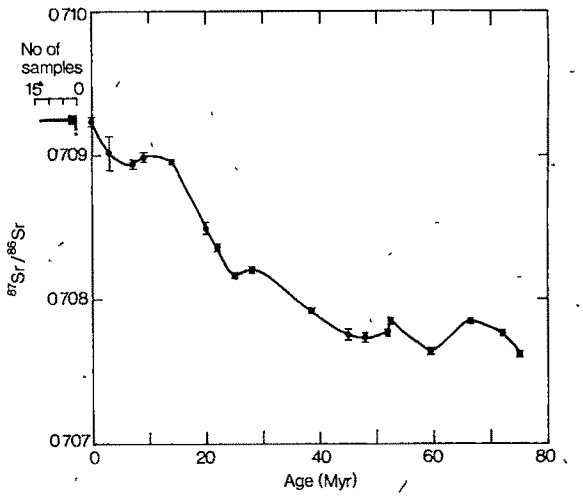


Fig. 1 Marine $^{87}Sr/^{86}Sr$ ratio since 75 Myr BP. Histogram is for 22 modern samples. National Bureau of Standards 987 $SrCO_3$ $^{87}Sr/^{86}Sr = 0.710275$, Eimer and Amend $SrCO_3$ $^{87}Sr/^{86}Sr = 0.708066$.

The results of this study are shown in Fig. 1 and Table 1. The form of the curve is in good agreement with the recently produced Burke curve[1]. A further aspect of Fig. 1 is that it seems probable that the seawater Sr isotope curve can be constrained to a higher precision than is implied by the earlier studies through careful sample selection and cleaning. This considerably strengthens the potential of the curve for stratigraphical correlation and absolute age determinations during periods showing the greatest rates of change of $^{87}Sr/^{86}Sr$ ratios (4×10^{-5} Myr^{-1}). Measurements of 24 Recent foraminifera samples (see histogram in Fig. 1) are identical to within 2.6×10^{-5} (2σ), indicating a notional resolution of ± 0.65 Myr (the practical limit of resolution is about ± 1.5 Myr). Thus, temporal variations in marine $^{87}Sr/^{86}Sr$ ratios can provide a high-resolution dating and palaeostratigraphical method for the Oligocene-Recent, with potential for other periods as well.

To examine the various processes that may control the marine $^{87}Sr/^{86}Sr$ curve, a model was constructed based on the marine geochemical cycle of Sr shown in Fig. 2. In the model, the major flux of dissolved Sr to the oceans is from river input, ~75% of which is derived from weathering of uplifted limestones with the remainder resulting from the weathering of silicate rocks[5,14]. Recycling also takes place through pore-water Sr diffusing into bottom waters from carbonate-rich sediments; a smaller flux is associated with sediments depleted in carbonate[15]. Sr isotope exchange takes place between basalt and seawater during circulation of seawater through fast-spreading mid-ocean-ridge hydrothermal systems[7-9]; no significant Sr mass transfer has been observed during this process. Neither Sr isotope exchange nor mass transfer is observed during lower temperature ridge-flank hydrothermal circulation[11] and the only significant sink for Sr in the modern oceans is biogenic calcium carbonate.

The present-day Sr fluxes are given in Fig. 2. Two values are given for the hydrothermal flux because of uncertainties over the proportions both of advective and convective heat flow at mid-ocean ridges and of advective heat loss associated with high-temperature ridge crest versus low-temperature ridge flank hydrothermal systems[13,16-18]. One value is derived from linking chemical data in hydrothermal vents with global heat and ^3He loss from the ocean crust[10-19]; the second value acknowledges the conclusions from recent geophysical and geochemical work (mass balance of alkalis and alkaline earths, Li in basalts, Ge/Si ratios) that the hydrothermal flux at ridge axes is considerably lower than originally estimated[12,13,20-23]. Although several studies have been made of the Sr isotope composition of river water[4,5,24], these have been limited to a few of the world's major rivers; a latest estimate from Canadian rivers[24], however, does suggest that the global average is likely to be ~0.711. By solving

Table 1 $^{87}Sr/^{86}Sr$ and Sr/Ca ratios in foraminiferal calcite from DSDP sites 21 and 357

Site	Depth (m)	Age (Myr)	$^{87}Sr/^{86}Sr \pm 2\sigma$	Sr/Ca ($\times 10^3$)
357	1	0	0.709242±27	1.35
357	11	3	0.709019±121	1.27
357	29	7	0.708942±28	1.15
357	39.5	9	0.708986±20	1.19
357	50	14	0.708955±14	1.19
357	105.5	20	0.708502±40	1.37
357	138	22	0.708373±15	1.26
357	194	25	0.708167±16	1.24
357	210	28	0.708207±27	1.23
257	258	38.5	0.707924±14	1.32
357	287.5	45	0.707758±37	1.38
357	380	48	0.707738±29	1.42
357	444.5	52	0.707772±18	1.17
357	467	52.5	0.707853±18	0.99
357	476.5	59.5	0.707641±22	1 12
21	92	66.5	0.707854±12	1.18
21	108	72	0.707771±16	1.08
21	128	75	0.707618±17	1.36

the steady state equations (see Fig. 2) for the riverine Sr isotope composition, using the data in Fig. 2, we obtain $^{87}Sr/^{86}Sr$ ratios of 0.7119 and 0.7102 (for the two different hydrothermal fluxes), both similar to the above value. However, the ratio required to balance the low hydrothermal flux yields a $^{87}Sr/^{86}Sr$ ratio for silicate weathering (0.7173) that is significantly closer to the average Sr isotope composition of aluminosilicate detritus in oceanic core top sediments (0.7166)[25] than does the ratio based on the high hydrothermal flux (0.7243).

Next, we considered temporal variations in the fluxes shown in Fig. 2. Oceanic crustal production rates are generally thought to have been higher 75 Myr BP than today[26,27]. If we assume that the hydrothermal flux is directly proportional to seafloor spreading rate[28], then variations in the hydrothermal Sr flux can be calculated. Three estimates of seafloor spreading rates were considered here: rates that were 60% higher 65 Myr BP than today[29], rates that were a maximum of 25% higher 75 Myr BP than today[30] and an intermediate rate[31] (corrected for subduction).

Because of the high concentration of Sr in marine carbonate, recycling of Sr from the weathering of uplifted limestones and diffusion of Sr from carbonate-rich sediment pore waters play an important part in the Sr marine geochemical cycle. From the mass half life of continental limestones[14] and the $^{87}Sr/^{86}Sr$ ratio of ancient seawater, Brass[5] calculated the average $^{87}Sr/^{86}Sr$ ratios derived from the weathering of uplifted limestones at intervals over the past 400 Myr. The $^{87}Sr/^{86}Sr$ ratio of pore waters diffusing into overlying waters from carbonate-rich sediments is equivalent to that of 18-Myr BP seawater[15]. Thus, similarly to Brass, we calculated the average $^{87}Sr/^{86}Sr$ ratio of this source against time.

The effects of carbonate recycling and varying hydrothermal fluxes on the marine $^{87}Sr/^{86}Sr$ ratio since 75 Myr BP, obtained by these methods, are shown in Fig. 3*a*. It is clear that these two controls cannot fully explain the observed Sr isotope variations. Moreover, the consequence of assuming the most rapid variations in spreading rates[29] is that they produce unrealistically high global atmospheric CO_2 levels, temperatures and sea levels 75 Myr BP[32] and, as noted above, recent evidence suggests that hydrothermal fluxes are lower than originally supposed. Hence, changes resulting from fluctuations in hydrothermal activity as well as from carbonate recycling appear to be minor. Thus, variations in the nature of the riverine Sr flux to the oceans must be a major control on temporal variations in the seawater $^{87}Sr/^{86}Sr$ ratio.

The lower $^{87}/^{86}Sr$ ratios in the past have been ascribed to lower river fluxes because of the decreased land area at that time[7,14]. However, the rate of continental weathering is a complex function of climatic and geographical variables[33]. Indeed, global CO_2 models suggest that the rate of weathering per unit area of continent was considerably higher 75 Myr BP than today because of the interplay of these factors[32]. It is likely, therefore, that the riverine Sr flux was at least equal to or higher than its present-day value. Thus, the low observed marine $^{87}Sr/^{86}Sr$ ratios relative to the model values (Fig. 3) must reflect one or more of the following factors: a higher internal/external continental drainage ratio 75 Myr BP; decreases in the proportion of Sr derived from the weathering of limestones since 75 Myr BP; increases in the average $^{87}Sr/^{86}Sr$ ratio derived from the weathering of silicate rocks since 75 Myr BP.

The increase in land area since 75 Myr BP[34] requires extreme assumptions to be made for a concurrent fall in the continental internal/external drainage ratio. The Sr derived from limestone weathering would have to have been 10 times higher than that now to account for the smallest model deviation from the observed marine $^{87}Sr/^{86}Sr$ ratios at 75 Myr BP (assuming a constant Sr flux magnitude and ratio from silicate weathering). Clearly, this mechanism alone cannot realistically account for the model deviations. These two arguments strongly suggest that

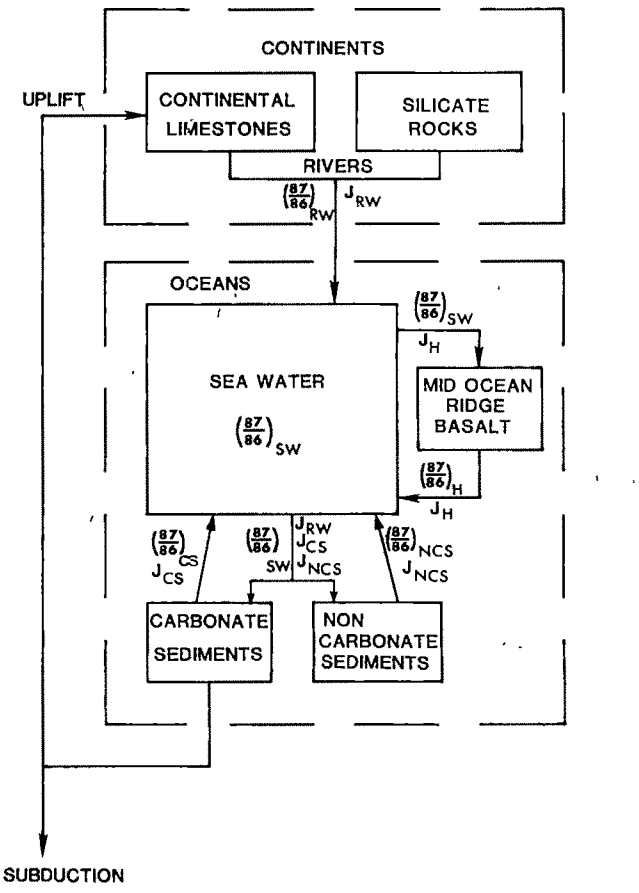


Fig. 2 Marine geochemical cycle of Sr. (J = flux; (87/86) = $^{87}Sr/^{86}Sr$ ratio of flux.) At steady state: $[87]_{SW} = ([87]_{RW}J_{RW} + [87]_H J_H + [87]_{CS}J_{CS} + [87]_{NCS}J_{NCS})/(J_{RW} + J_H + J_{CS} + J_{NCS})$; $[86]_{SW} = ([86]_{RW}J_{RW} + [86]_H J_H + [86]_{CS}J_{CS} + [86]_{NCS}J_{NCS})/(J_{RW} + J_H + J_{CS} + J_{NCS})$; where SW = seawater, RW = river water, H = hydrothermal isotope exchange, CS = carbonate sediment pore waters, NCS = non-carbonate sediment pore waters. Magnitudes of present-day fluxes (mol yr^{-1}) are: $J_{RW} = 2.5 \times 10^{10}$ (ref. 37), $J_{NCS} = 4.0 \times 10^8$ (ref. 15), $J_{CS} = 3.0 \times 10^9$ (ref 15), $J_H = 1.2 \times 10^{10}$ (ref. 10) $= 0.38 \times 10^{10}$ (ref. 11). $^{87}Sr/^{86}Sr$ ratios of present-day fluxes are: $(87/86)_{NCS} = 0.7064$ (ref 15), $(87/86)_{CS} = 0.7087$ (ref. 15), $(87/86)_H = 0.7040$ (refs 8, 9), $(87/86)_{SW} = 0.70924$ (this work). Sensitivity of model to uncertainties in above values; resulting uncertainty in calculated $(87/86)_{SW}$ is 2×10^{-4} for uncertainty in J_{RW} and 1.4×10^{-4} for uncertainty in $(87/86)_H$; all other variables produce negligible uncertainties in $(87/86)_{SW}$ or are considered in the text.

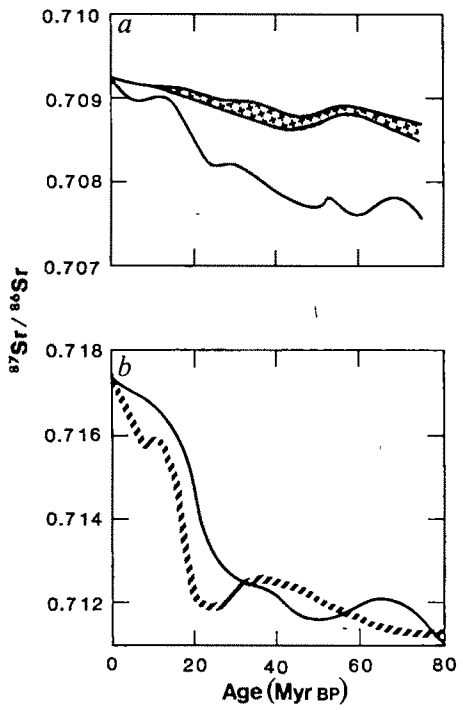


Fig. 3 *a*, Variations in the marine $^{87}Sr/^{86}Sr$ ratio due to changes in rates of hydrothermal activity and carbonate recycling. Results using an intermediate rate of change in J_H (ref. 31) are shown (high and low rates of change in J_H (refs 29, 30) show similar patterns). The shaded area gives the range in $^{87}Sr/^{86}Sr$ ratios calculated from high and low values of J_H (refs 10, 11) and the bottom curve shows the measured values of $^{87}Sr/^{86}Sr$ for sea water The model curves were calculated at 10-Myr intervals. *b*, Variations in the $^{87}Sr/^{86}Sr$ ratio derived from the weathering of silicate rocks: the shaded area shows qualitative variations in glacial intensity (after Armstrong[6])

changes in the $^{87}Sr/^{86}Sr$ ratio derived from the weathering of silicate rocks must play a dominant role in varying the marine $^{87}Sr/^{86}Sr$ ratio.

Figure 3*b* shows the changes in silicate $^{87}Sr/^{86}Sr$ ratios (at a constant riverine flux magnitude) required to produce the observed marine $^{87}Sr/^{86}Sr$ ratios, assuming a low hydrothermal flux and smaller changes in seafloor spreading rates. The most notable feature of the curves in Fig. 3*b* is the sharp rise in $^{87}Sr/^{86}Sr$ ratios from 25 Myr BP to the present, with relatively constant Sr isotope ratios before this time. This feature correlates well with two important variables in geological history. First, the similarity to the qualitative curve of glacial activity over the past 75 Myr BP is striking, providing some circumstantial support for Armstrong's[6] hypothesis that increased glacial action has affected the marine $^{87}Sr/^{86}Sr$ ratio by both exposing and increasing the weathering rates of ancient shield areas; these terrains have high $^{87}Sr/^{86}$ ratios[35]. Second, there is overwhelming evidence to suggest that during the late Cretaceous and early Cenozoic total worldwide volcanism was greater than at any subsequent time (see, for example, ref. 36). The past 30 Myr are marked by the Alpine and Himalayan orogenies. These widespread folding events resulted in the uplifting and accelerated erosion of rocks characterized by high $^{87}Sr/^{86}Sr$ ratios. Thus, the rise in the $^{87}Sr/^{86}Sr$ ratio derived from silicate weathering since 25 Myr BP may reflect a recovery from the previous era when weathering of young basaltic volcanics was more dominant.

We conclude, therefore, that whilst hydrothermal fluxes and carbonate recycling are of great importance in determining the absolute value of the marine $^{87}Sr/^{86}Sr$ ratio at any one instant, the major control over the variations in the ratio over the past 75 Myr has been changes in the isotope composition of Sr derived from silicate rock weathering.

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1 Burke, W H *et al Geology* **10**, 516-519 (1982)
2 Peterman, Z E, Hedge, C. E & Tourtelot, H A *Geochim. cosmochim. Acta* **34**, 105-120 (1970)
3 Dasch, E J & Biscaye, P E *Earth planet Sci Lett* **11**, 201-204 (1971)
4 Veizer, J & Compston, W *Geochim cosmochim Acta* **38**, 1461-1484 (1974)
5 Brass, G W *Geochim cosmochim Acta* **40**, 721-730 (1976)
6 Armstrong, R L *Nature phys Sci* **230**, 132-133 (1971)
7 Spooner, E T C *Earth planet Sci Lett* **31**, 167-174 (1976)
8 Albarede, F, Michard, A, Minster, J F & Michard, G *Earth planet Sci Lett* **55**, 229-236 (1981)
9 Elderfield, H & Greaves, M J *Geochim cosmochim Acta* **45**, 2201-2212 (1981)
10 Edmond, J M. *et al Earth planet Sci Lett* **46**, 1-18 (1979)
11 Bender, M L, Hess, J, Schilling, J G & Waggoner, D G *Trans Am. geophys Un* **63**, 1014 (1982)
12 Mottl, M J *Bull geol Soc Am* **94**, 161-180 (1983)
13 Sleep, N H *Hydrothermal Processes at Seafloor Spreading Centers* (Plenum, New York, 1983)
14 Garrels, R M. & MacKenzie, F T *Evolution of Sedimentary Rocks* (Norton, New York, 1971)
15 Elderfield, H & Gieskes, J M *Nature* **300**, 493-497 (1982)
16 Wolery, T J & Sleep, N H *J Geol* **84**, 249-275 (1970)
17 Anderson, R N, Langseth, M G & Sclater, J G *J geophys Res* 3391-3409 (1977)
18 Sclater, J G, Parsons, B & Jaupart, C. J *J geophys Res* **86**, 11535-11552 (1981)
19 Jenkins, W J, Edmond, J M & Corliss, J B *Nature* **272**, 156-158 (1978)
20 Hart, S R & Staudigel, H *Earth planet Sci Lett* **58**, 202-212 (1982)
21 Seyfried, W E, Janecky, D R & Mottl, M J *Geochim cosmochim Acta* **48**, 557-569 (1984)
22 Sleep, N H, Morton, J L, Burns, L E & Wolery, T J *Hydrothermal Processes at Seafloor Spreading Centers* (Plenum, New York, 1983)
23 Stoffyn-Egli, P & Mackenzie, F T *Geochim cosmochim Acta* **48**, 859-872 (1984)
24 Wadleigh, M A, Veizer, J & Brooks, C *Geochim cosmochim Acta* (in the press)
25 Dasch, E J *Geochim cosmochim Acta* **33**, 1521-1552 (1969)
26 Pitman, W C & Talwani, M *Bull. geol Soc. Am* **83**, 619-646 (1972)
27 Hays, J D & Pitman, W C *Nature* **246**, 18-82 (1973)
28 Delaney, M L thesis, Massachusetts Inst Technology (1983)
29 Pitman, W C *Bull geol Soc Am* **89**, 1389-1403 (1978)
30 Parsons, B *J geophys Res* **87**, 289-302 (1982)
31 Southam, J R & Hay, W W *J Geophys Res* **82**, 3825-3842 (1977)
32 Berner, R A, Lasaga, A C & Garrels, R M *Am J Sci* **283**, 641-683 (1983).
33 Holland, H D *The Chemistry of the Atmosphere and the Oceans* (Wiley, New York, 1978)
34 Vail, P R, Mitchum, R M & Thompson, S *Am Ass Petrol Geol* **26**, 83-97 (1978)
35 McCulloch, M T & Wasserburg, G J *Science* **200**, 1003-1011 (1978)
36 Ronov, A B *Geochim. Int* **13**, 172-195 (1976)

Pine wood origin for pitch from the *Mary Rose*

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The raising of the wreck of King Henry VIII's flagship (AD 1509–45), the *Mary Rose*, from the bed of the Solent was a unique event, notable as a significant technical achievement in naval architectural history and as a source of well-preserved Tudor relics. Preservation is attributed to the anaerobic environment prevailing within the sediment during much of the period of burial and, in some cases, to the large quantities of pitch that permeated the ship and many of the relics. Although the function of many relics is usually easily recognized, their means of construction and the nature and origin of the materials used in their manufacture are often much less obvious. We report here the chemical analysis of six samples of pitch from the *Mary Rose*. Computerized gas chromatography/mass spectrometry (CGC/MS) and infrared spectroscopy have been used to 'fingerprint' the pitches and compare them with modern-day tars and pitches derived from wood, coal, peat and petroleum. Diterpenoid hydrocarbons, methyl dehydroabietate and dehydroabietic acid were found in similar proportions to 'Stockholm tar (good-quality pine tar obtained by the destructive distillation of *Pinus sylvestris*), so providing conclusive evidence for the derivation of the *Mary Rose* pitches from pine wood.

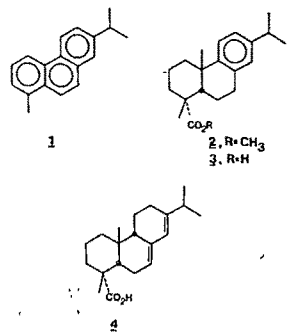
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Until recently, chemical archaeological investigations have been concerned largely with the inorganic components of, for example, building materials, pottery, coins and weapons[1]. This apparent bias towards inorganic materials probably arises from their greater likelihood of survival over archaeological time compared with organic substances. Many inorganic materials remain largely unchanged after tens or even hundreds of years of weathering, whereas organic-based plant and animal products are rapidly decomposed unless protected from the degradative effects of moisture, oxygen and sunlight.

Historically, the use of tars and pitches as protective hydrophobic coatings is well established. However, to our knowledge no detailed chemical investigations have been performed to determine the origins or means of manufacture of such materials. Related organic chemical archaeological studies include GC analysis of animal fats from peat bogs and Eskimo dwelling sites[2,3], plant and animal residues from caves and pottery[4], a ^{13}C-NMR investigation of non-metallic seals[5], an infrared study of tar from a thirteenth century Norwegian shipwreck[6] and investigations of natural resins of art and archaeology, including GC and GC/MS studies[7,8].

Six samples of pitch and pitch-impregnated materials from the *Mary Rose* were selected for analysis. These included pitch from barrels found in the hold, 'luting' from the main deck, caulking from between the timbers of the outer frame of the ship, tarred anchor cable and a tarry solid (probably intrusive) found in a box of longbows. Table 1 lists these samples and their reference numbers.

GC and GC/MS analysis of the components of the *Mary Rose* pitches eluting in hexane, toluene and dichloromethane (DCM), and those obtained by esterification of the acid fraction, revealed in every case relatively simple mixtures of compounds. The hexane-eluting components of the pitch from the barrels produced the most complex patterns (Fig. 1*a*, *b*), with eight components comprising >90% of the total volatile components (calculated by integration of the reconstructed ion current (RIC) peak areas). The mass spectra of components 1–8 in Fig. 1 displayed molecular ions (M^+) at m/z 256 ($C_{19}H_{28}$), 254 ($C_{19}H_{26}$), 256 ($C_{19}H_{28}$), 252 ($C_{19}H_{24}$), 238 ($C_{18}H_{22}$), 220 ($C_{17}H_{16}$), 234 ($C_{18}H_{18}$) and 248 ($C_{19}H_{20}$), respectively. Comparison of these with reference spectra[9] indicated that the compounds were alkyl-substituted tricyclic diterpenoid hydrocarbons, the major component (peak 7 in Fig. 1) being retene (compound 1).



1 2 3 4

Further GC/MS analyses revealed the presence of methyl dehydroabietate (compound 2, m/z 314, $C_{21}H_{30}O_2$) as the major (>80%) ester component of the LC fractions from the *Mary Rose* samples. This latter compound was also the major constituent (>90%) of the methylated acid fraction, indicating that the principal acid is dehydroabietic acid (compound 3).

Diterpenoid compounds of this type have long been known to be major constituents of conifer resins[10,11], hence these CGC/MS data provide conclusive evidence that the *Mary Rose* pitches were produced from pine trees. As such compounds are not appreciably observed in peat and coal tars or petroleum bitumens, these can be discounted as potential sources. GC and CGC/MS of fractionated Stockholm tar produced a very similar distribution of hydrocarbons (Fig. 1*c*) to those of the barrels of pitch from the *Mary Rose*. Methyl dehydroabietate was also the major ester constituent and dehydroabietic acid again the major component of the acid fraction.

Table 1 *Mary Rose* tars and pitches and other materials

Sample	Appearance and odour	Origin
'Tarred' rope (MR AC)	20 strands 0.5–20 cm long, impregnated with tar	Anchor cable
'Pitch' (82 S 1298)	Shiny black conchoidally fractured fragments with strong 'tarry' odour	Barrel in the hold
'Luting' (MR 82 S1274/12)	Small pieces of damp tarred wood with a strong 'tarry' odour	Main deck
Caulking (MR 82 S126)	Rope-like material composed of hair fibres impregnated with tar	Between the timbers forming the outer frame
'Tar' (82 S1296/7)	Thick black tarry material with 'tarry' odour	Barrel in the hold
Solid (81 S300)	Soft dark-brown solid, smelling strongly 'tarry'	Inside chest/box of longbows (probably intrusive)

Bracketed codes were used by the Mary Rose Trust to document the samples. For simplicity the term 'pitch' has been used in the text but the terms 'tar' and 'pitch' are not synonymous. Tar is the initial pyrolysate, which may then be thickened to pitch by boiling to drive off volatiles and effect a degree of further polymerization. Pitches of different softening points are obtainable in this way, but the present study does not attempt to distinguish between them or tars.

The remarkable similarities between the composition of the *Mary Rose* samples and 'Stockholm tar' clearly indicate that the sixteenth century and present-day tars and pitches are of the same generic type. These chemical data are in agreement with the historical and geographical evidence for the probable sources of the *Mary Rose* pitch[12].

Although the hydrocarbon distributions of the homogeneous pitch contained in the barrels are obviously similar to modern 'Stockholm tar', those obtained from the pitches already in their intended site of application (for example, 'luting', caulking and anchor rope) are somewhat different and variable[12]. Nevertheless, they do comprise only the characteristic diterpenoid hydrocarbons referred to above. The differences in distribution may reflect varying degrees of microbial alteration, weathering or water washing during the ship's 36-yr active life and subsequent 437-yr submergence and burial. Alternatively, they may reflect differing means of preparation before use.

It is notable that none of the abietic acid (compound 4) commonly observed in pine resins[10,11] was detected in either the *Mary Rose* samples or the 'Stockholm tar'. Presumably, the abietic acid present in the wood is thermally dehydrogenated and, to a lesser extent, decarboxylated during the retorting process, to produce the mixture of diterpenoid hydrocarbons, methyl dehydroabietate and dehydroabietic acid described above.

Today Stockholm tar or similar tars are imported into the United Kingdom from Russia, Eastern Europe, Scandinavia, China, America and India. At the time of the *Mary Rose*, it is known that trade developed between Russia and England, and amongst the first cargos were tar and pitch[13,14]. The similarity in chemical composition between the *Mary Rose* samples and the modern Russian Stockholm tar is consistent with the *Mary Rose* pitch having originated from one such cargo, but a local source of manufacture cannot be ruled out.

We have recently examined further pitch samples from an excavation in York (early medieval) and an Etruscan shipwreck (600 BC) discovered off the Island of Giglio, Italy. Both exhibited infrared spectra similar to those of Stockholm tar and *Mary Rose* pitch. CGC/MS analyses have also shown them to

contain abundant diterpenoid compounds. Clearly, pine wood derived tars and pitches have been in use as maritime waterproofing agents for well over 2,000 yr.

This investigation demonstrates the power of GC and GC/MS in the analysis of the organic components of archaeological materials and illustrates the scope of the problems awaiting the application of modern analytical methodology.

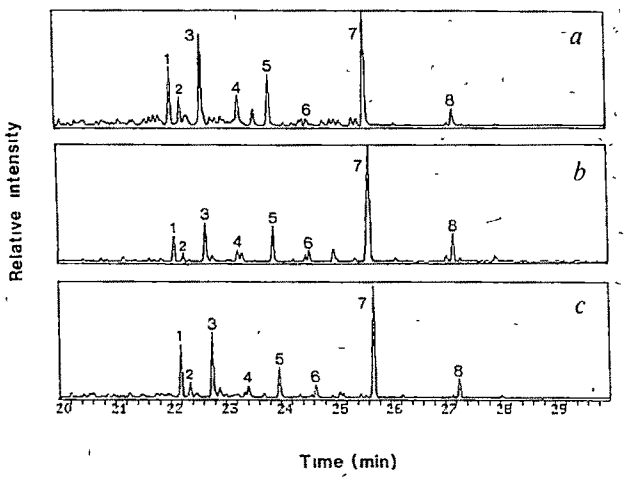


Fig. 1 Partial gas chromatograms showing the hydrocarbon fractions of: *a*, *Mary Rose* tar (82 S 1296/7); *b*, *Mary Rose* pitch (82 S 1298), *c*, present-day 'Stockholm tar' obtained by the destructive distillation of pine wood of the genus *Pinus*. Peak assignments of the alkyl-substituted tricyclic diterpenoid hydrocarbons were by CGC/MS using a Finnigan 9610 GC linked to a Finnigan 4000 quadrupole MS. Data acquisition and processing were performed using a Finnigan INCOS 2300 data system. Peak identities are: 1 = 19-norabieta-8,11,13-triene (M^+ 256, $C_{19}H_{28}$); 2 = unknown (M^+ 254, $C_{19}H_{26}$); 3 = 18-norabieta-8,11,13-triene (M^+ 256, $C_{19}H_{28}$); 4 = simonellite (?, M^+ 252, $C_{19}H_{24}$), 5 = 1,2,3,4-tetrahydroretene (M^+ 238, $C_{18}H_{22}$); 6 = mixture, predominantly ethylmethylphenanthrene (M^+ 220, $C_{17}H_{16}$); 7 = retene (compound 1) (M^+ 234, $C_{18}H_{18}$); 8 = unknown higher homologue of retene (M^+ 248, $C_{19}H_{20}$). Where the *Mary Rose* pitches were neither adhered to, nor incorporated into, any other materials, no extraction was necessary. In the cases of the caulking, 'luting' and rope samples, their associated pitch required extraction before analysis, achieved by placing the sample in an elution tube plugged with pre-washed cotton wool and washing with DCM (~200 ml). Extraction was continued until no further coloration appeared in the solvent washings. Removal of the DCM by rotary evaporation rendered the pitch extract free from particulate matter. Preliminary examination of the *Mary Rose* pitches and extracts was performed by infrared spectrophotometry as either melted films or as solutions in DCM. The infrared spectra of all the *Mary Rose* samples were similar, displaying absorptions assignable to carboxylic acid groups and aliphatic and aromatic moieties, and are very different from those recorded for petroleum bitumens and coal tars, but show some similarity to that obtained from a peat tar. The most striking similarities were observed between the *Mary Rose* samples and commercial 'Stockholm tar', whose spectrum is comparable in almost every respect (cm^{-1} and absorption intensity) to those of the *Mary Rose* pitches. Further comparisons required detailed molecular analysis by GC and GC/MS In preparation for this, aliquots (~1 g) of the various tars and pitches were fractionated by liquid chromatography (LC, alumina, Al_2O_3) using gradient elution (100 ml each of *n*-hexane, toluene, DCM, ethyl acetate and methanol). Isolation of the acidic components of a *Mary Rose* tar (82 S 1298) and 'Stockholm tar' was achieved by shaking aliquots (0.5 g) of tar dissolved in DCM (5 ml) with aqueous potassium hydroxide (KOH, 5%, 4 × 5 ml) Neutralization (5 M HCl) of the aqueous extract, and extraction (DCM, 3 × 20 ml) produced the acids, which were converted to their corresponding methyl ester derivatives using ethereal diazomethane. Analyses were performed on a Carlo Erba 4160 GC, using on-column injection. The column was 25 m × 0.3 mm i.d. OV-1 coated flexible silica capillary column (Chrompak). Hydrogen was the carrier at 100 cm s^{-1} and the GC oven temperature was programmed over 50–300 °C at 6 °C min^{-1}. The GC runs were acquired on a VG Analytical Minichrom data system[15].

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1 Goffer, Z *Archaeological Chemistry* (Wiley, Chichester, 1980)
2 Thornton, M D, Morgan, E D & Celoria, F *Science and Archaeology* **2–3**, 20–24 (1970)
3 Morgan, E D, Titus, L, Small, R J & Edwards, C *Arctic* **36**, 356–360 (1983)
4 Rottlander, R C A & Schlichtherle, H *Archaeophysika* **10**, 260–267 (1979)
5 Cassar, M, Robins, G V, Fletton, R A & Alstin, A *Nature* **303**, 238–239 (1983)
6 Plahter, U *Saertrykk Fra, Universitetets Oldsaksamlings Arbok*, 217–219 (1969)
7 Mills, J S & White, R *Stud Conserv* **22**, 12–31 (1977), *Natn Gallery tech Bull* **6**, 3–18 (1982)
8 White, R & Castle, S A *Britannia* **9**, 383–392 (1978)
9 Simonett, B R T. thesis, Univ Bristol (1975)
10 Thomas, B R in *Organic Geochemistry–Methods and Results* (eds Eglinton, G & Murphy, M T J) 599–618 (Springer, Berlin, 1969)
11 Thomas, B R in *Phytochemical Phylogeny* (ed Harborne, J B) Ch 4, (Academic, New York, 1970)
12. Forbes, R J *Bitumen and Petroleum in Antiquity*, 1–31 (Brill, Leiden, 1936)
13 Danischewsky, M I *Post-Medieval Archaeology* Vol 9, 77–78 (Society for Post-Medieval Archaeology, 1975)
14 Meiggs, R *Trees and Timber in the Ancient Mediterranean World* (Clarendon, Oxford, 1982)
15 Jerman, K thesis, Univ Bristol (1984)

Filamentous microfossils from the 3,500-Myr-old Onverwacht Group, Barberton Mountain Land, South Africa

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The Swaziland Supergroup, Barberton Mountain Land, South Africa, has long been regarded as a promising location for the Earth's oldest fossils because it includes some of the most ancient well-preserved sedimentary rocks, many of which contain carbonaceous matter. Although there have been numerous reports of microfossils from Swaziland Group rocks[1–7], the biogenicity of most of the structures has been questioned[8–10]. Although some of the organic spheroids are probably biogenic[10,11], the best early Archaean simple spheroids are generally regarded as 'possible microfossils'[10] because organic spheroids may form abiotically in several ways[12]. The discovery of less-simple biological morphologies is therefore important in establishing the existence of early life forms in the early Archaean. Uniformly-sized curving filaments, especially tubular ones, are difficult to explain as anything other than the fossil remains of filamentous organisms. Here we report the discovery of numerous filaments from two different stratigraphical positions in the 3,500-Myr-old Onverwacht Group of the Swaziland Supergroup. Their morphologies and abundance provide convincing evidence for the existence of bacteria- or cyanobacteria-like organisms on the Earth during the early Archaean. This supports recent reports of similar filamentous microfossils from 3,500-Myr-old rocks from Western Australia[13].

The Onverwacht Group consists of ultramafic and mafic lavas interbedded with minor felsic tuffs and sedimentary units, including carbonaceous cherts. Nd–Sm dating of komatiitic flows in both lower and upper parts of the Onverwacht Group yields an age of 3,500 Myr[14]. A minimum age of 3,300 Myr for

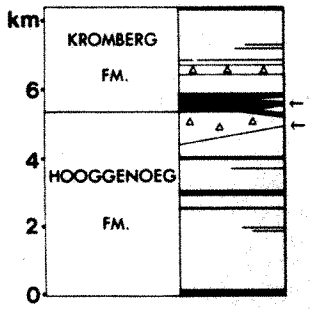


Fig. 1 Stratigraphical column of the Hooggenoeg and Kromberg Formations, upper Onverwacht Group, Swaziland Supergroup, Barberton Mountain Land. Unornamented mafic and ultramafic volcanic rocks; △, volcaniclastic rocks; solid shading, chert. Arrows indicate position of fossiliferous samples.

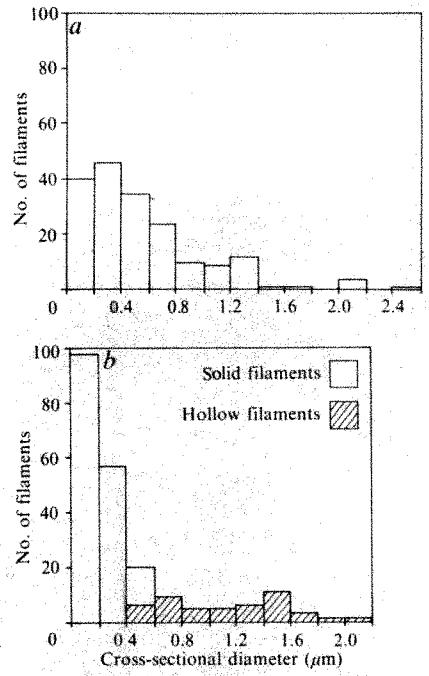


Fig. 2 Size-frequency distribution of filamentous microstructures. *a*, Hooggenoeg sample (185 microstructures); *b*, Kromberg sample (216 microstructures).

the Onverwacht Group is provided by K–Ar dating of low-grade regional metamorphism which has affected the entire sequence[15].

The oldest unit in which we have identified filamentous microstructures is located near the top of the Hooggenoeg Formation in the upper half of the Onverwacht Group (Fig. 1). The structures occur in a black and white banded chert that is interbedded with thin silicified volcaniclastic layers at the base of a thick sequence of felsic tuffs and conglomerates. It crops out ~2 km north of the Komati Gorge section described by Viljoen and Viljoen[16]. The Hooggenoeg sample locality is 26°00′30″S, 31°00′05″E. It is exposed in a south-west-facing fault escarpment of the major ridge that generally trends north-east. A native trail crosses the ridge in a saddle south of the outcrop. Carbonaceous matter in the black chert bands occurs as fine laminae, as granules draped by laminae and as layers of irregularly shaped grains. The carbonaceous matter is identified by its petrographic characteristics—its brown-black colour and lack of crystallinity. Furthermore, whole rock analyses yield a total organic carbon content of 0.04%. Within this layer, the filamentous structures are thread-like or cylindrical and non-septate (Fig. 3*a*), with cross-sectional diameters ranging from <0.2 μm to 2.6 μm (Fig. 2*a*). The filaments are the same colour as surrounding organic matter, so they are probably partially carbonaceous. In addition, fine pyrite grains along the filaments are visible using reflected light microscopy. In a single carbonaceous layer ~2.5 cm thick, 185 individual filaments were noted in a total area of thin section 20-cm square.

The second sample containing carbonaceous filaments was collected in the immediately overlying Kromberg Formation. The filaments occur in a section of black and white banded cherts interlayered with flat- and cross-laminated clastic carbonates ~380 m above the base of the formation in the Komati Gorge section of Viljoen and Viljoen[16]. (The Kromberg sample locality is 26°01′42″S, 30°00′05″E. The prominent outcrop of banded chert is exposed on the path along the south bank of Komati River, ~2 km upriver from the footbridge.) The fossiliferous sample consists of alternating bands of brown-black carbonaceous chert and colourless translucent chert. The total organic carbon content of the rock is 0.11%. The carbonaceous chert layers, varying from 0.3 to 4 cm thick, have undulating tops and bottoms and are made up of billowy brown-black organic matter interlayered with fine carbonaceous laminae, both permineralized with silica. Within the fine laminae are mudstone clasts and pellets of organic matter. Many of the filaments are associated with the fine laminae, but some are within the cloudy organic matter. There are at least two distinct populations of filaments present. The most abundant filaments are threadlike (Fig. 3*b*, *f*), ranging in diameter from 0.1 to 0.6 μm and lengths from 10 to 150 μm (Fig. 2*b*). The other population consists of tubular filaments (Fig. 3*b*, *c* and *d*) with cross-sectional diameters ranging from 1.4 to 2.2 μm and lengths from 10 to 150 μm (Fig. 2*b*). Most filaments in this size range appear non-septate, but a few exhibit slight constrictions at intervals of ~1 μm. Based on the same transmitted and reflected microscopic criteria described for the Hooggenoeg sample, the filaments appear to be composed of carbonaceous matter, and in some cases admixed pyrite grains. The filaments are much more abundant in this sample than in the Hooggenoeg sample, with more than 200 individuals located in a 2.4-cm square area of thin section.

The age of the filaments, only slightly more than 1,000 Myr younger than the Earth itself, requires that the structures be appraised carefully with respect to their biogenicity. First, the characteristics of the rocks containing the filaments must be considered. All of the relevant features of the rocks are consistent with biogenicity of the filaments. The rock units in which the fossiliferous samples occur are clearly sedimentary units. The Hooggenoeg sample is in a 3.5-m-thick section of interbedded black and white cherts and flat- and cross-laminated sand and volcanic accretionary lapilli. The cherts are part of the sequence, with conformable contacts with over- and under-lying sand units. The sedimentary sequence overlies a komatiitic lava flow that has a brecciated weathered top. The Kromberg sample is from cherts that have a sedimentary lower contact with a pillowed lava flow. The flow has a brecciated top and signs of alteration due to submarine weathering. The section of cherts is conformably overlain by flat- and cross-laminated carbonates[17]. Traced laterally, the chert unit maintains its position in the sequence and is an original part of the sedimentary sequence. The rocks have undergone only low-grade metamorphism[16] and thus have not been heated sufficiently to destroy carbonaceous fossils. The black or brown-black colour of the organic matter in the rocks indicates that it has experienced heating conditions consistent with low-grade metamorphism[18]. The similar thermal history of the carbonaceous matter and the surrounding rocks strengthens the argument for a similar time of formation for the carbonaceous rocks and the surrounding sediments and volcanic flows. Carbon isotope analyses on black-and-white-banded cherts from the same Kromberg section yield $^{13}C_{PDB}$ values of −34.5 and −33.9 ± 0.2‰. These values are close to values reported for both Archaean and Proterozoic fossiliferous stromatolitic samples[18]. Perhaps most importantly, the microscopic texture of the carbonaceous cherts is like that of stromatolitic cherts, although larger-scale stromatolitic relief is lacking. The fine

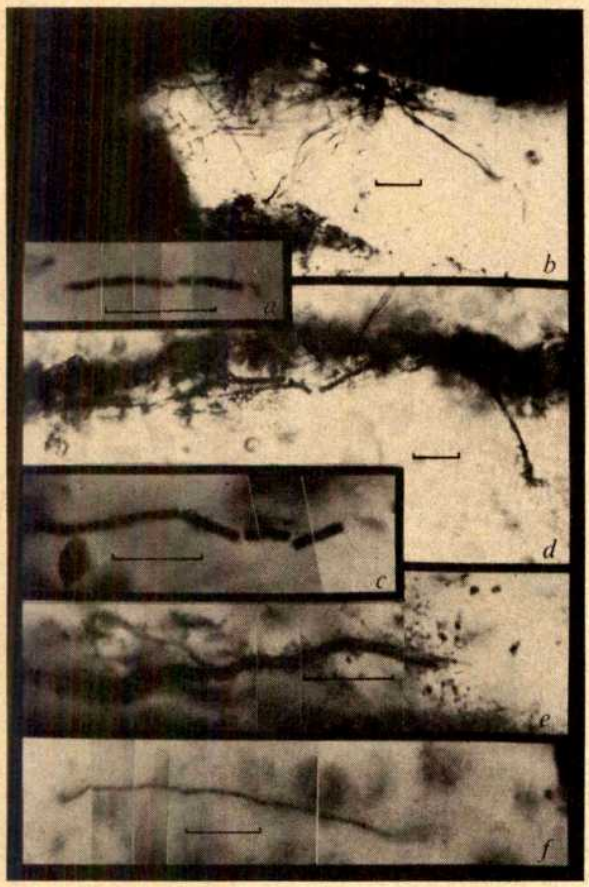


Fig. 3 Filamentous microfossils: *a*, cylindrical microfossil from Hooggenoeg sample; *b*, threadlike and tubular filaments extending between laminae, Kromberg sample; *c*, *d*, *e*, tubular filaments oriented subparallel to bedding, Kromberg sample; *f*, threadlike filament flattened parallel to bedding, Kromberg sample.

crinkly apparently-coherent carbonaceous laminae are similar to those formed by microbial mats populated by filamentous organisms that precipitate and/or trap sediment between successive growth layers.

Characteristics of the filaments themselves indicate that they are both indigenous to the original deposit and biological in origin. The filaments are located far from any weathering surfaces or veins and have a similar colour to surrounding organic matter, indicating a similar thermal history. The filaments are, therefore, unlikely to be recent endolithic organisms[19]. The filaments cut across crystal domains and are thus not merely concentrations of organic matter at crystal boundaries. Both the size and the shape of the carbonaceous filaments are similar to those of modern filamentous bacteria and cyanobacteria and Proterozoic filamentous fossils interpreted to be the remains of cyanobacteria or bacteria[20–24]. The filaments are abundant and have a narrow size range. Each filament has a fairly constant diameter along its length. The spatial relationship of the filaments to lamination, generally either subparallel or perpendicular (Fig. 3), is like that of filaments in modern fossil mats constructed by phototrophic organisms[25]. Many of the thread-like filaments that are aligned parallel to bedding appear ribbon-like, as if they had been compressed during sediment compaction (Fig. 3*f*). Such flattening of microfossils has been described from both shales[26] and cherts[27]. Solid minerals or recent contaminants would be unlikely to show such flattening. The apparent composition of the filaments, carbonaceous matter and fine pyrite, is consistent with biogenicity. Filamentous microfossils consisting of pyrite grains have been reported from both the Beck Springs Dolomite[28] and the Gunflint Formation[21] and haematite-coated cylindrical microfossils are found in the Belt Supergroup[29]. Furthermore, cross-sectional views of larger filaments in the Kromberg sample show that they are hollow tubes, not solid mineral cylinders (R. J. Horodyski, personal communication. Horodyski has noted solid pyritic cylinders of abiogenic origin in cherts of the Belt Supergroup, Northwestern Montana.) The presence of pyrite along the filaments means either that the pyrite replaced the organic matter making up the walls of degrading filaments, or that the pyrite or a mineral it replaced was precipitated on the living organisms. Note that the apparent diameters of the filaments may reflect pyrite crystal size, or thickness of the pyrite coating, and not original filament size.

The filaments reported here represent the most probable biological microstructures yet reported from the Swaziland Supergroup and are similar in age to carbonaceous filamentous microfossils described from rocks in the eastern Pilbara block, Western Australia[13]. They are also the same age as the oldest known stromatolites, reported from Western Australia[30,31]. Carbonaceous cherts with organic mat-like textures are not uncommon in the Swaziland Supergroup. Their abundance, the above described microfossils and the occurrence of both microfossils and stromatolites in Western Australia provide compelling evidence for the existence of mat-forming organisms in early Archaean shallow water environments.

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1. Pflug, H. *Econ. Geol. Res. Unit. Witwatersrand Univ. Inf. Circ.* No. 28 (1966).
2. Barghoorn, E. S. & Schopf, J. W. *Science* **152,** 758–763 (1966).
3. Schopf, J. W. & Barghoorn, E. S. *Science* **156,** 508–512 (1967).
4. Nagy, B. & Nagy, L. A. *Nature* **223,** 1226–1228 (1969).
5. Engel, E. A. J. *et al. Science* **161,** 1005–1008 (1968).
6. Muir, M. D. & Grant, P. R. in *The Early History of the Earth* (ed. Windley, B. F.) 595–604 (Wiley, New York, 1976).
7. Muir, M. D. & Hall, D. O. *Nature* **252,** 376–378 (1974).
8. Schopf, J. W. *A. Rev. Earth planet. Sci.* **3,** 213–249 (1975).
9. Schopf, J. W. *Origins Life* **7,** 19–36 (1976).
10. Schopf, J. W. & Walter, M. R. in *Earth's Earliest Biosphere* (ed. Schopf, J. W.) 214–239 (Princeton University Press, New Jersey, 1983).
11. Knoll, A. H. & Barghoorn, E. S. *Science* **198,** 396–398 (1977).
12. Folsome, C. A., Allen, R. D. & Ichinose, M. K. *Precambr. Res.* **2,** 263–275 (1975).
13. Awramik, S. M. Schopf, J. W. & Walter, M. R. *Precambr. Res.* **20,** 357–374 (1983).
14. Hamilton, P. J., Evensen, M. M., O'Nions, R. K., Smith, H. S. & Erlank, A. J. *Nature* **297,** 298–300 (1979).
15. DeWit M. J., Hart, R., Martin, A. & Abbott, P. *Econ. Geol.* **77,** 1783–1802 (1982).
16. Viljoen, R. P. & Viljoen, M. J. *Geol. Soc. S. Afr. Spec. Pap.* **2,** 113–151 (1969).
17. Lowe, D. R. & Knauth, L. P. *J. Geol.* **85,** 699–723 (1977).
18. Hayes, J. M., Kaplan, I. R. & Wedeking, K. W. in *Earth's Earliest Biosphere* (ed. Schopf, J. W.) 93–134 (Princeton University Press, New Jersey, 1983).
19. Cloud, P. & Morrison, K. *Precambr. Res.* **9,** 81–91 (1979).
20. Brock, T. D. *Biology of microorganisms* (Prentice-Hall, Englewood Cliffs, 1979).
21. Barghoorn, E. S. & Tyler, S. A. *Science* **147,** 563–577 (1965).
22. Schopf, J. W. *J. Paleontol.* **42,** 651–688 (1968).
23. Awramik, S. M. & Barghoorn, E. S. *Precambr. Res.* **5,** 121–142 (1977).
24. Knoll, A. H. *J. Paleontol.* **56,** 755–790 (1982).
25. Monty, C. L. V. *Ann. Soc. Geol. Belg.* **90,** 55–100 (1967).
26. Horodyski, R. J. & Bloeser, B. *Science* **199,** 682–684 (1978).
27. Hofmann, H. J. *J. Paleontol.* **50,** 1040–1073 (1976).
28. Gutstadt, A. M. & Schopf, J. W. *Nature* **223,** 165–167 (1969).
29. Horodyski, R. J. *Precambr. Res.* **2,** 215–254 (1975).
30. Lowe, D. R. *Nature* **284,** 441–443 (1980).
31. Walter, M. R., Buick, R. & Dunlop, M. S. R. *Nature* **284,** 443–445 (1980).

Opposite motivational effects of endogenous opioids in brain and periphery

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Many psychoactive drugs, including the opiates, have been shown to have paradoxical reinforcing effects[1,2]. Opiates produce positive reinforcing effects when they are paired with visual and textural environmental stimuli in rats[3,4], yet, at similar doses and over the same routes of administration, produce aversive effects, as shown when they are paired with taste stimuli[5]. Similarly, in human, the positive reinforcing effects of opiates are well known to addicts and recreational drug users, yet patients receiving opiates as analgesics often report nauseous reactions. At present there is no evidence to differentiate between the neural substrates that mediate these opposite motivational effects. We now report an initial step in the resolution of this paradox by demonstrating that endogenous and exogenous opioids produce positive reinforcing effects through an action on brain opiate receptors, and aversive effects through an action on peripheral opiate receptors (especially in the gut).

The microinjection of morphine directly into certain specific brain sites can produce positive reinforcing effects similar to those seen after systemically administered drug[1]. The central dose required to produce these effects is lower than that needed to produce similar effects after systemic administration, suggesting that the primary population of receptors mediating the positive reinforcing effects is localized in the brain[6]. Recently, a large population of opiate receptors has been found on primary sensory neurones in the peripheral nervous system[7]. Local application of opiates in the periphery produces receptor-mediated hyperalgesic or irritation effects[8]. These results suggest that opiates, and by analogy endogenous opioids, produce positive reinforcing effects in the brain and aversive effects in the periphery. To test this hypothesis, we studied the motivational effects of specific opioid receptor antagonists which do or do not cross the blood–brain barrier.

Using a place-preference paradigm[3], separate groups of drug-naïve rats were administered (subcutaneously (s.c.) or intraperitoneally (i.p.)) various doses of naltrexone or its quaternary derivative methylnaltrexone, which does not cross the blood–brain barrier effectively[9]. Briefly, each rat received four drug injections spaced equally over 8 days, and was immediately confined after each injection to a visually and texturally distinctive environment for 30 min. On the 4 alternating days when no opiate antagonist injection was given, each rat was injected with

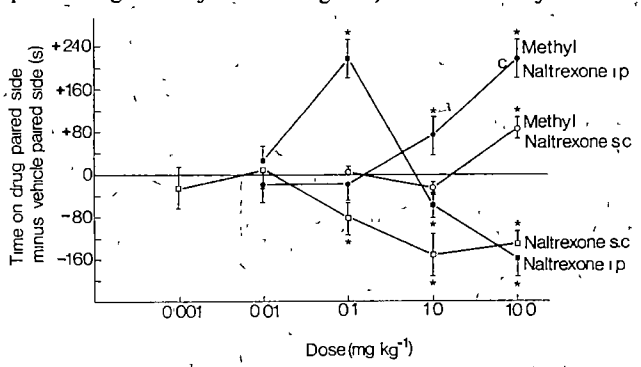


Fig. 1 Effects of various doses of naltrexone and methylnaltrexone (s.c. and i.p.) in the place-conditioning paradigm. Each point represents the mean ± s.e.m. for eight rats ($*P < 0.05$). Overall analyses of variance on the data from i.p. (0.01–10 mg kg^{-1}) and s.c. (0.1–100 mg kg^{-1}) injections revealed most importantly significant three-way interactions of time in the drug-versus-saline paired environment with drug and with dose ($F_{3,42} = 19.57$ (i.p.), $F_{2,28} = 12.91$ (s.c.), $P < 0.005$).

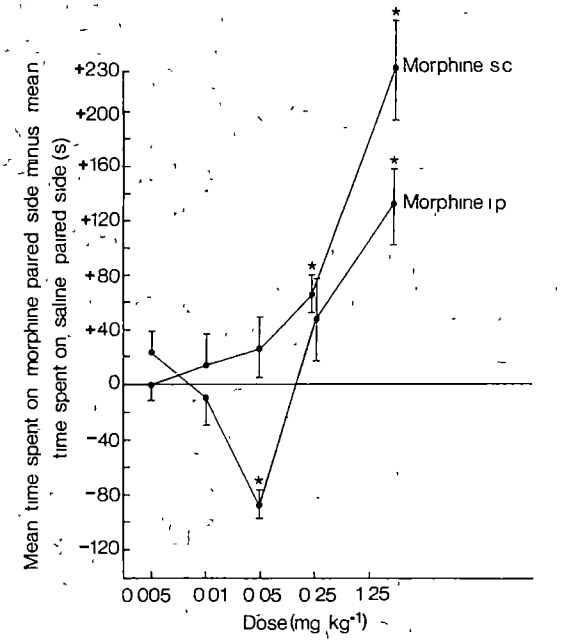


Fig. 2 Effects of various doses of morphine (s.c. and i.p.) in the place-conditioning paradigm. Each point represents the mean ± s.e.m. for eight rats ($*P < 0.05$).

saline vehicle and confined for 30 min to another separate and different distinctive environment[3]. Order of injections and drug-paired environment were counterbalanced within groups. On day 9, each uninjected rat was tested by recording the amount of time the rat spent in each of the two previously paired distinctive environments when given a 10-min period to explore both environments freely.

Figure 1 shows that, regardless of the route of administration, increasing doses of naltrexone were aversive whereas increasing doses of methylnaltrexone were positively reinforcing. The results suggest that the place aversions observed were due to an antagonism by the antagonist of endogenous opioid peptides acting on central opiate receptors. This suggestion is based on the fact that most opiate receptors are located in the brain and therefore the net effects of naltrexone, which can cross the blood–brain barrier, are mainly caused by its binding to central receptors. Similar results have been reported previously using another opiate antagonist, naloxone[3]. The preferences produced by methylnaltrexone, which does not effectively cross the blood–brain barrier, must result primarily from its binding to peripheral opiate receptors and its blockade of peripheral endogenous opioid actions.

Interestingly, one anomalous result occurred at 0.1 mg kg^{-1} naltrexone i.p., but not s.c., which produced a place preference ($F_{1,7} = 17.23$, $P < 0.05$). We hypothesized that this was the result of a local block of aversive opiate effects in the gut without significant central action at this low dose. This implies that the peripheral aversive effects of opiates are mediated primarily by receptors in the gut. Based on this hypothesis, we predicted that a low dose of morphine, given i.p., should produce aversion. Figure 2 shows that 0.05 mg kg^{-1} of morphine (i.p.) produced significant place aversions ($F_{1,7} = 27.47$, $P < 0.05$); the same dose administered s.c. had no effect. Higher doses of morphine (s.c. and i.p.) were positively reinforcing in the place-preference paradigm; doses below 0.05 mg kg^{-1} had no effects. Because the vagus nerve carries sensory information from the gut to the brain, we predicted further that subdiaphragmatic vagotomies should abolish the observed effects at the low doses of i.p. morphine and naltrexone (0.05 mg kg^{-1} morphine place aversions and 0.1 mg kg^{-1} naltrexone place preferences), but should not abolish the opposite motivational effects seen at higher i.p. doses (1.25 mg kg^{-1} morphine place preferences and 10.0 mg kg^{-1} naltrexone place aversions). Figure 3 confirms these predictions. Vagotomy (verified at death by measuring

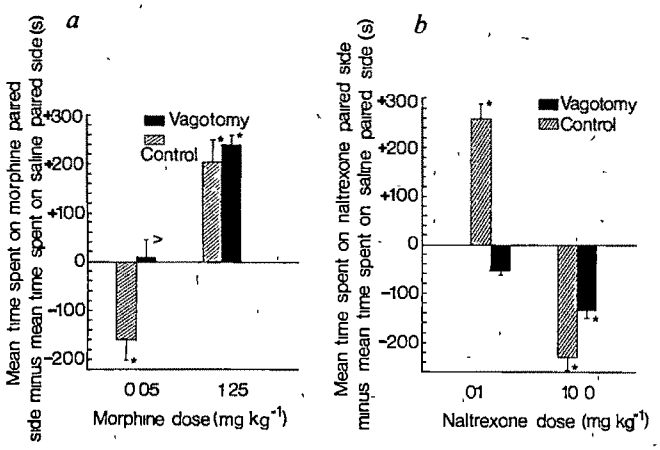


Fig. 3 *a*, Effects of vagotomy on the low-dose i.p. morphine place aversions and higher-dose i.p. place preferences. *b*, Effects of vagotomy on the low-dose i.p. naltrexone place preferences and higher-dose i.p. place aversions. Bars are means ± s.e.m. for 7–12 rats. * Indicates significant place conditioning ($P < 0.05$). Overall analyses of variance on the data from control and vagotomized animals injected with morphine and naltrexone revealed significant two-way interactions of control versus vagotomized animals with time in drug-versus-saline paired environment ($F_{1,14} = 17.88$, $P < 0.05$ (naltrexone); $F_{1,22} = 6.05$, $P < 0.05$ (morphine)).

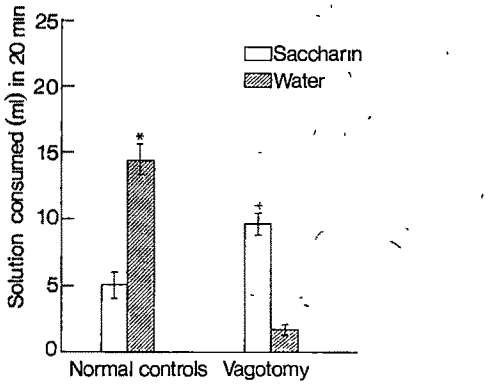


Fig. 4 Effects of vagotomy on the aversions elicited by high-dose i.p. morphine injections in the conditioned taste aversion paradigm. Bars are means ± s.e.m. for 7–10 rats. * Indicates a significant difference between saccharin and water consumption ($P < 0.05$).

food retention in the stomach[10]) blocked the 0.05 mg kg^{-1} morphine-induced place aversions and the 0.1 mg kg^{-1} naltrexone-induced place preferences. No significant effects of vagotomies were observed on the 1.25 mg kg^{-1} morphine place preferences nor on the 10.0 mg kg^{-1} naltrexone place aversions. These data support the hypothesis that the aversive properties of opiates are mediated by means of gut opiate receptors.

We tested this hypothesis further by investigating the effects of vagotomy on the aversive effects of opiates in the conditioned taste aversion paradigm[5], where only aversive effects are seen even with higher doses of morphine. Fluid-deprived vagotomized ($n = 7$) and control ($n = 10$) rats received five 20-min presentations (spaced equally over 10 days) of 0.1% saccharin solution followed immediately by a morphine injection (15 mg kg^{-1}, i.p.), and on the 5 alternating days normal water paired with saline vehicle injection. Overall analyses of variance on a final drug-free, 20-min, two-bottle choice test revealed a significant two-way interaction of vagotomy versus control with saccharin versus water consumption ($F_{1,18} = 18.35$, $P < 0.05$). The control rats demonstrated an aversion for the saccharin flavour previously paired with morphine, whereas the vagotomized and morphine-injected rats showed the normal preference for saccharin (Fig. 4) seen in uninjected animals. These results again suggest that the vagus nerve carries information concerning the aversive properties of opioids from the gut to the brain.

In conclusion, endogenous and exogenous opioids acting on peripheral receptors (especially in the gut) produce aversive or nauseous effects, whereas opioids acting on central brain receptors produce positive reinforcing or euphoric effects. The present results suggest that the adverse effects of opiates experienced in human patients receiving opiates for analgesia might be minimized by blocking peripheral opiate receptors. These data also provide the first steps in analysing how the organization of the nervous system places constraints on the learning of associations between the two different, anatomically specific motivational effects of opioids and stimuli in different sensory systems.

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1 Cappell, H & Leblanc, A E *Addictive Behav* **1**, 55–64 (1975)
2 Wise, R A, Yokel, R A & Dewitt, H *Science* **191**, 1273–1275 (1976)
3 Mucha, R F, van der Kooy, D, O'Shaughnessy, H & Bucenicks, P. *Brain Res* **243**, 91–105 (1982)
4 Weeks, J R *Science* **138**, 143–144 (1962)
5 van der Kooy, D & Phillips, A G *Pharmac. Biochem Behav* **6**, 637–641 (1977).
6 van der Kooy, D, Mucha, R F, O'Shaughnessy, M & Bucenieks, P *Brain Res* **243**, 107–117 (1982)
7 Ninkovic, M, Hunt, S P & Gleave, J R W *Brain Res* **241**, 197–206 (1982)
8 van der Kooy, D *Neurosci. Lett Suppl* **10**, S498 (1982)
9 Valentino, R J, Herling, S, Woods, J H, Medzihradsky, F & Merz, H *J. Pharmac* **217**, 652–659 (1981)
10 Martin, J R, Rogers, R C, Novin, D. & van der Weele, D A *Bull. psychon Soc.* **10**, 291–294 (1977)

Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase

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Interaction of ligands with 'Ca^{2+}-mobilizing' receptors is thought to result in the generation of two second messengers, inositol trisphosphate and diacylglycerol, from a common substrate, phosphatidylinositol 4, 5-bisphosphate (PtdIns(4, 5)P_2) (refs 1, 2), a component of plasma membranes[3,4]. It is not known how the occupation of such receptors is translated into the activation of the catalytic unit polyphosphoinositide (PPI) phosphodiesterase, and then to cellular activation, but our recent experiments suggest that GTP regulatory proteins may be involved. In mast cells, non-hydrolysable analogues of GTP introduced and then trapped in the cytosol are able to substitute for external ligands in inducing exocytosis, a well-defined Ca^{2+}-dependent process[5], suggesting that guanine nucleotide regulatory proteins may act by stimulating the catalytic activity of the PPI phosphodiesterase. We now provide evidence that mast cell secretion is inhibited by internalized neomycin, a compound known to interact with PPI[6]. We also show that the PPI phosphodiesterase of human neutrophil plasma membranes can be activated simply by adding GTP analogues in the presence of concentrations of Ca^{2+} that pertain in unstimulated cells. These findings strongly support the idea that the coupling factor linking receptor and PPI phosphodiesterase is a guanine nucleotide binding protein analogous to those involved in the activation and inhibition of adenylate cyclase[7].

Mast cells, when transiently permeabilized in the presence of the GTP analogue GTP-γ-S, secrete histamine when Ca^{2+} is added subsequently to the external medium[5]. To test the possibility that PPI hydrolysis is required for the action of GTP-γ-S, we investigated the effects of neomycin, which prevents the hydrolysis of PPIs. Figure 1 shows that when neomycin at 10–100 μM is loaded into the cytosol with GTP-γ-S (50 μM), Ca^{2+}-dependent secretion is abolished. Neomycin inhibits

Table 1 GTP-γ-S-dependent hydrolysis of polyphosphoinositides and corresponding increase in inositol phosphates

	Radioactivity (c.p.m.) in:	
Polyphosphoinositides	PtdIns(4)P	PtdIns(4,5)P_2
Control	21,579±453	19,854±397
GTP-γ-S	17,362±359	13,112±470
Loss of label	4,217	6,742
Inositol phosphates	$InsP_2$	$InsP_3$
Control	7,245±155	14,450±1,845
GTP-γ-S	11,746±344	21,072±370
Increase in label	4,501	6,672

Abbreviations: PtdIns(4)P, phosphatidylinositol 4-phosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate, $InsP_2$, inositol bisphosphate; $InsP_3$, inositol trisphosphate. Human peripheral neutrophils were prepared as described previously[14]. The cells (10^8 per ml) were preincubated in a balanced salt solution[14] containing ^{32}P-phosphate (100 μCi ml^{-1}) for 90 min to prelabel the phospholipids, including the polyphosphoinositides. A light membrane fraction enriched in plasma membranes was prepared as described previously[25]. The plasma membrane suspension was suspended in 20 mM PIPES buffer containing 150 mM KCl, *p*H 6.8, at a protein concentration of 3–4 mg ml^{-1}. Samples (500 μl) were transferred to tubes containing GTP-γ-S and Ca-EGTA buffer (3 mM EGTA final) in an equal volume to give final concentrations of GTP-γ-S and Ca^{2+} of 100 μM and 100 nM, respectively. After 10 min at 37 °C, the reaction was terminated by the addition of 3.75 vol chloroform/methanol/concentrated HCl (100:200:5). This mixture was allowed to stand for 30 min and then separated into two phases by the addition of 1.25 vol each of chloroform and water. The lower (chloroform) phase was retained for lipid analysis and the upper (aqueous) phase retained for the determination of inositol phosphates Phospholipids were separated by TLC on oxalate-impregnated silica gel-H-coated plates using chloroform/methanol/acetone/acetic acid/water (40:13:15:12:8) and the radioactive spots located by autoradiography. Areas containing PtdIns4P and PtdIns(4,5)P_2 were scraped and the lipids hydrolysed in 72% perchloric acid at 180 °C for 1 h. The sample was made up to 10 ml in water and counted by Cerenkov radiation. For the analysis of the water-soluble inositol phosphates, the upper (aqueous) phase was neutralized, made up to 10 ml with water and run on Dowex 1 (formate form) as described previously[26]. Results (c p.m.) are expressed as the means (±s.d.) of triplicate determinations from a single experiment. Similar results were obtained in two other experiments. Samples of labelled membranes were also treated with Ca^{2+} (1 mM) alongside each experiment for comparison and on this particular occasion, Ca^{2+}-dependent activation of the phosphodiesterase caused loss of PtdIns4P (55%) and PtdIns(4,5)P_2 (65%) compared with GTP-γ-S-activated loss of PtdIns4P (20%) and PtdIns(4,5)P_2 (34%)

secretion only when presented to the cells together with ATP^{4-}, which causes cell permeabilization[8] and so permits the drug entry to the cytosol. This result suggested that PPI hydrolysis is an integral step in the activation of cells loaded with non-hydrolysable GTP analogues and that PPI phosphodiesterase is subject to control by a guanine nucleotide binding protein. To test this possibility, we used the PPI phosphodiesterase of human neutrophils. We have described recently a plasma membrane PPI phosphodiesterase in human and rabbit neutrophil membranes which can be activated in the presence of Ca^{2+} (ref. 9). Like the red cell enzyme[10], the neutrophil enzyme is not under the control of cytosol Ca^{2+} because Ca^{2+} is required in the millimolar range. The possibility must therefore be considered that the enzyme is controlled by some other so far unidentified component.

GTP-γ-S can activate the neutrophil enzyme (Table 1) in the conditions of Ca^{2+} homeostasis pertaining in resting cells. The hydrolysis of PPI yields the water-soluble products inositol bis- and trisphosphate. The loss of label from PPI is fully accounted for by the appearance of label in the inositol phosphates (Table 1). Data for Ca^{2+} activation of the enzyme are also included for comparison (see Table 1 legend). Our earlier experiments included Mg^{2+} (1 mM) in the incubation mixtures, but it soon became clear that this leads to hydrolysis of the inositol phosphates, with the implication that Mg^{2+}-requiring phosphatases

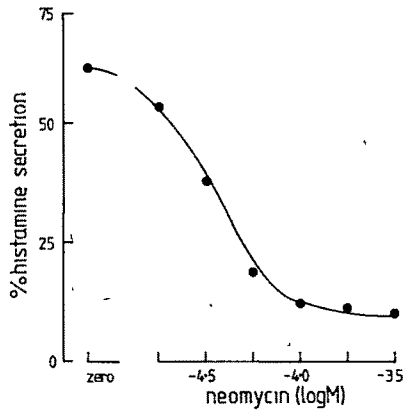


Fig. 1 Inhibition by neomycin of Ca^{2+}-dependent histamine secretion from GTP-γ-S-loaded mast cells. Exogenous solutes were loaded into the cytosol of rat mast cells as described previously[5]; they were treated with ATP (3.16 μM), GTP-γ-S (50 μM) and neomycin (concentrations as indicated on the abscissa). This treatment with ATP in the absence of divalent cations causes the formation of lesions in the plasma membrane and allows the other solutes present to penetrate into the cytosol. At 6 min, $MgCl_2$ (5 mM) was added to close the membrane lesions and so trap the loaded GTP-γ-S and neomycin in the cytosol. After 15 min further incubation, exocytotic secretion was triggered by the addition of Ca^{2+} (5 mM). The cells were quenched with ice-cold phosphate-buffered saline 10 min later, and secreted histamine was measured as described previously[8]. Neomycin at concentrations up to 300 μM had no effect on the histamine assay. The results illustrated are representative of four independent experiments

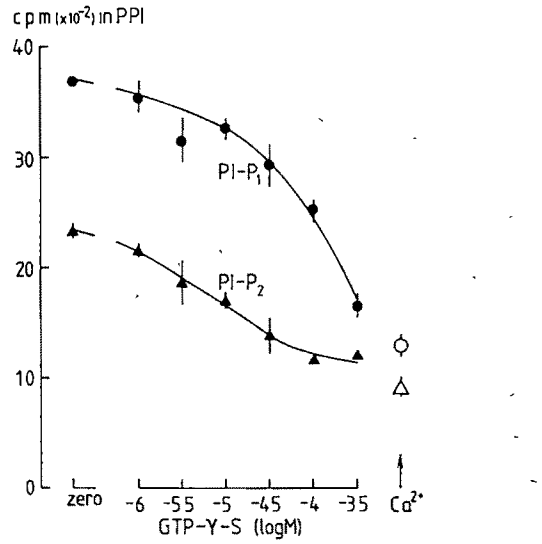


Fig. 2 Concentration–effect relationship of GTP-γ-S-activated polyphosphoinositide phosphodiesterase in neutrophil membranes Experimental details are the same as outlined in Table 1 legend except that the incubations were performed using 100 μl of membrane suspension added to 100 μl of buffer containing Ca-EGTA (100 nM Ca^{2+}), 1 mM Mg^{2+} (final) and GTP-γ-S as indicated For comparison, data for PPI phosphodiesterase activation by 1 mM Ca^{2+} are also shown (open symbols). The results illustrated are representative of four separate experiments. Results are expressed as c.p.m. in the individual polyphosphoinositides, determinations were made in triplicate. Error bars denote s.d. Circles denote PtdIns4P (PI-P_1) and triangles denote PtdIns(4,5)P_2 (PI-P_2).

are present; such phosphatases have been identified recently in the red cell[11]. Mg^{2+} was therefore excluded subsequently from experiments in which water-soluble derivatives were to be analysed. There was no indication that Mg^{2+} is required for the action of GTP-γ-S on the enzyme, and it has little effect on the inositol lipid phosphomonoesterase[9] and no effect on PPI phosphodiesterase[9].

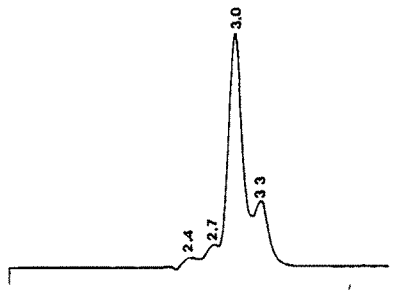


Fig. 3 HPLC analysis of commercial GTP-γ-S. GTP-γ-S (10 μl of 1 mM) was injected into a Bondepak cartridge-type column and the sample run isocratically as described previously[3,14]. Chart recording speed was set at 20 mm min^{-1}. GTP-γ-S and IDP were obtained from Boehringer Mannheim and all other nucleotides from Sigma. GppNHp was obtained from both sources and produced similar HPLC profiles. Elution times (min) for the various nucleotides were: ATP, 4.95; GTP, 2.98; GDP, 3 27; GMP, 4.47; ADP, 5 7; GTP-γ-S, 3.0 (68%) and 3.3 (20%, probably GDP); GppNHp, 3.07 (65%) and 3.42 (30%); $GppCH_2p$, 3.04; IDP, 3.32.

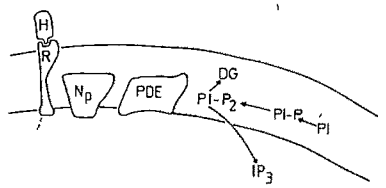


Fig. 4 Schematic description of events that occur when hormone binds to its receptor and results in transmembrane signalling. Interaction of hormone (H) with receptor (R) allows a guanine nucleotide binding protein (N_p) to exchange bound GDP for GTP. This generates an activated species of N_p which activates the catalytic unit, polyphosphoinositide phosphodiesterase (PDE). The hydrolysis of phosphatidylinositol bisphosphate (PI-P_2) generates two second messengers—diacylglycerol (DG) to activate protein kinase C and inositol trisphosphate to release Ca^{2+}—which then together mediate cellular responses. The supply of the substrate (PI-P_2) is limited by the activity of the inositol lipid kinases which sequentially phosphorylate phosphatidylinositol (PI) to phosphatidylinositol 4-phosphate (PI-P) and then to PI-P_2.

Activation of PPI phosphodiesterase was dependent on the concentration of GTP-γ-S in the range 1–300 μM (the highest concentration tested) (Fig. 2). Activity was already apparent at 1 μM and at higher concentrations approached activities that can be achieved by millimolar concentrations of Ca^{2+} (ref. 9). However, we stress that these results refer to the added compound, obtained from commercial sources. The guanine nucleotides are inherently unstable; in particular, there is a tendency towards deamination to yield inosine homologues[12]. Analysis by HPLC reveals that GTP-γ-S (Fig. 3) and GppNHp (see Fig. 3 legend) are always very impure; the impurity detected is most probably GDP in the case of GTP-γ-S (Fig. 3) and remains unidentified in the case of GppNHp. The proportions of the individual components did not alter during 8 months' storage. However, the HPLC method[13,14] does not resolve guanine from their equivalent inosine nucleotides, and so we cannot be certain of the actual concentrations required for activation of the enzyme, although we do know that the onset of activation occurs at concentrations <1 μM. In five separate experiments, 1 μM GTP-γ-S hydrolysed 10% (±2) (s.e.m., $n = 5$) of phosphatidylinositol 4-phosphate and PtdIns(4, 5)P_2.

We also tested the specificity of the GTP analogues, but the uncertainties involved in the actual concentrations present prevented us from quantifying their relative potencies. All compounds were tested at a nominal 100 μM. GDP, GDP-β-S, ATP, ATP-γ-S and AppNHp were inactive, whereas GTP-γ-S, GppNHp and $GppCH_2p$ caused activation. The effect of GTP was erratic, probably because of hydrolysis by a nonspecific nucleotidase known to be present in neutrophil plasma membranes[15].

The results described here provide firm evidence that PPI phosphodiesterase can be activated by analogues of GTP, and so by inference may be subject to control by the binding of GTP at a receptor-controlled guanine nucleotide binding protein (N-protein) in its cellular environment (Fig. 4). In this it is similar to adenylate cyclase, although there are also important differences. In particular, whereas the availability of substrate to adenylate cyclase is effectively inexhaustible, the substrate for PPI phosphodiesterase is an endogenous membrane lipid, strictly limited by the inositol lipid kinases, and thus perhaps under independent control (S.C., J. Lamb and E. Zanders, in preparation). Further, there are two products of phosphodiesterase action, one of which (diacylglycerol) is retained in the organic phase of the membrane, while the other (inositol trisphosphate) is released into the cytosol.

Binding of ligand to receptor leads to the binding of GTP and activation of N-protein. Different receptors are likely to interact with separate but related families of N-proteins; N_i, N_s and transducin have already been described[16]. We designate the putative N-protein which is involved in the activation of PPI phosphodiesterase as N_p; an inhibitory counterpart to N_p analogous to N_i probably also exists. The activated N_p is then responsible for switching on the PPI phosphodiesterase as shown in Fig. 4.

These ideas are further supported by the recent finding that islet activating protein (purified from pertussis toxin) can inhibit mast cell and neutrophil secretion induced by both internalized GTP analogues[17] and receptor-directed agonists[18]. This suggests that N_p is susceptible to ADP-ribosylation and thus inhibition similar to N_i and transducin.

The precedent for involving N-proteins was set initially by the adenylate cyclase system[7] and this role has now been extended not only to hormone receptor action, but also to the biosynthesis of proteins[19]. Other examples include the light-induced activation of cyclic GMP phosphodiesterase[20] and the activation of protein kinase and cyclic AMP phosphodiesterase by insulin[21,22]. The protein products of the *ras* oncogene family also have a binding site for GTP, again implying a role for N-protein[23,24]. Thus, the activation of PPI phosphodiesterase shares with other receptor and non-receptor signal-transducing systems a common mechanism of control involving a family of related but specific N-proteins.

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1 Michell, R H. *Trends pharmac Sci* **8**, 263-265 (1983)
2 Berridge, M J *Biochem J* **220**, 345-360 (1984)
3 Downes, P & Michell, R H *Cell Calcium* **3**, 467-502 (1982).
4 Cockcroft, S, Taylor, J. & Judah, J D. *Biochim biophys Acta* (in the press)
5 Gomperts, B D *Nature* **306**, 64-66 (1983)
6 Schacht, J *J Lipid Res* **19**, 1063-1067 (1978)
7 Rodbell, M *Nature* **284**, 17-22 (1980)
8 Bennett, J P, Cockcroft, S & Gomperts, B D *J Physiol, Lond* **317**, 335-345 (1981)
9 Cockcroft, S, Baldwin, J M & Allan, D *Biochem J* **221**, 477-482 (1984)
10 Downes, C P & Michell, R H *Biochem J* **202**, 53-58 (1982)
11 Downes, C P, Mussat, M C & Michell, R H *Biochem J* **203**, 169-177 (1982)
12. *Remarks Concerning the Stability of GDP, GTP and dGTP* (Boehringer Mannheim technical leaflet, 1984)
13 Anderson, Q S & Murphy, R C *J Chromat* **121**, 251-262 (1976)
14 Cockcroft, S *Biochim biophys Acta* **795**, 37-46 (1984)
15 Hyslop, P A *et al FEBS Lett* **166**, 165-169 (1984)
16 Houslay, M D. *Trends biochem Sci* **9**, 39-40 (1984)
17 Nakamura, T & Ui, M *FEBS Lett* **173**, 414-418 (1984)
18 Okajima, F. & Ui, M *J biol. Chem* **259**, 13863-13871 (1984)
19 Kaziro, Y *Biochim biophys Acta* **505**, 95-127 (1978)
20 Stryer, L *Cold Spring Harb Symp quant Biol* **48**, 841-852 (1983)
21 Heyworth, C M, Rawal, S & Houslay, M D *FEBS Lett* **154**, 87-91 (1983)
22 Heyworth, C M, Wallace, A V & Houslay, M. D *Biochem J.* **214**, 99-110 (1983)
23. McGrath, J P, Capon, D. J., Goeddel, D V & Levinson, A D *Nature* **310**, 644-649 (1984)
24 Newbold, R. *Nature* **310**, 628-629 (1984)
25. Bennett, J P, Caswell, A H, Cockcroft, S & Gomperts, B D *Biochem. J* **208**, 801-808 (1982)
26 Downes, C P & Michell, R H *Biochem J* **198**, 133-140 (1981)

Antigen-specific interaction between T and B cells

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It is well known that B cells require T-cell help to produce specific antibody. Classic experiments suggested that antigen-specific helper T cells interact with antigen-specific B cells via an antigen 'bridge'[1,2], the B cells binding to one determinant on an antigen molecule (the 'hapten'), while the T cells at the same time recognize another determinant (the 'carrier'). T-helper cells bind specifically to antigen-presenting cells (APC), which have picked up and processed the appropriate antigen, and this interaction, like the interaction of T-helper cells with specific B cells, is restricted by products encoded by the major histocompatibility complex (*MHC*)[3-5]. Whereas conventional APC such as macrophages display no binding specificity for antigen, B cells have clonally distributed antigen-specific surface immunoglobulin receptors which would be expected to enhance their capacity to present antigen to T cells [6,7]. These findings are difficult to reconcile with the simple 'antigen bridge' mechanism of interaction, because it is hard to visualize how the bimolecular complex (processed antigen plus MHC molecule) on the APC surface can resemble the trimolecular complex (antigen bound to surface immunoglobulin plus MHC molecule) on the B-cell surface. To address this problem, we have cloned and immortalized human antigen-specific B cells with Epstein–Barr virus (EBV) and analysed their interaction with T-cell clones specific for the same antigen. We report here that surface immunoglobulin is indeed involved in the uptake and concentration of antigen, allowing specific B cells to present antigen to T cells with very high efficiency. However, the antigen must first be internalized and processed by specific B cells and it is then presented to T cells in an MHC-restricted manner indistinguishable from that characteristic of conventional APC.

Three B-cell clones producing IgG antibody against tetanus toxoid (TT) were isolated by EBV-induced transformation of peripheral blood B cells from an immune donor. The capacity of these B cells to bind antigen was tested by saturation analysis using ^{125}I-labelled TT. The double reciprocal plot of the bound versus free antigen showed an affinity constant of 5×10^7–2×10^8 l mol^{-1}, the number of binding sites varying from 2×10^4 to 10^5 per cell (see Table 1). The affinity for antigen of the secreted antibody was comparable within a factor of 3 to that of the B cells (data not shown). By contrast, B cells cloned from the same donor but producing IgG of an irrelevant specificity bound only very limited amounts of TT and showed no tendency for saturation. Furthermore, binding of ^{125}I-TT to TT-specific B cells was inhibited by unlabelled TT whereas binding to non-specific B cells was not (Table 1). These data indicate that specific B cells bind soluble antigen with a high affinity equal to the affinity of the antibody they produce, while B cells not specific for TT (nonspecific) bind much lower, although still measurable, amounts of antigen in an immunoglobulin-nonspecific way.

We compared the ability of TT-specific and nonspecific B cells to present TT or an unrelated antigen to T cells. The dose of antigen in culture was titrated for any T/B-cell combination and the T-cell proliferative response was assessed by thymidine incorporation. We found that both specific and nonspecific B cells were able to present native TT to TT-specific T-cell clones (Fig. 1*a*–*c*). However, while presentation by nonspecific B cells required high concentrations of antigen in culture (Fig. 1*c*), TT-specific B cells could trigger T-cell proliferation in the presence of 10^4-fold lower antigen concentrations (10^{-11}–10^{-12} M, Fig. 1*a*,*b*). We have obtained similar results in 12 T/B-cell combinations using 4 different T-cell clones and 3 different specific B-cell clones.

Table 1 Antigen binding by antigen-specific B cells

B-cell clone	^{125}I-TT bound per 10^6 cells (c.p.m.)	k (l mol^{-1})	No. of sites per cell
11.3	43,529 (1,039)	5×10^7	10^5
8.5	26,834 (593)	2×10^8	2×10^4
4.2	21,067 (912)	5×10^7	5×10^4
7.1	841 (730)	ND	ND

TT was labelled with ^{125}I by the chloramine-T method. B cells from three TT-specific EBV-transformed B-cell clones (11.3, 8.5 and 4.2) or from a clone producing an immunoglobulin of irrelevant specificity (7.1) were incubated with increasing concentrations of ^{125}I-TT in 200 µl phosphate-buffered saline containing 0.5% bovine serum albumin and 15 mM NaN_3. After 1 h on ice, the cells were pelleted and 100 µl of supernatant was collected. The cells were resuspended, washed twice by centrifugation through a cushion of fetal calf serum (FCS) and the radioactivity bound was determined. c.p.m. represent the amount of ^{125}I-TT bound near the saturation point of the curve. Numbers in parentheses indicate c.p.m. bound at the same concentration of labelled TT in the presence of a 100-fold excess of cold TT. k, The affinity of TT binding by B cells, and the number of binding sites were calculated from the double reciprocal plot of the saturation curve as described previously[12]. ND, the value was not determined because saturation could not be achieved.

Table 2 Antigen presentation by specific B cells requires intracellular antigen processing

Expt 1: Treatment of B cells before washing	Added in culture	^{3}H-thymidine incorporated (c.p.m.) by T-cell clone KT2	KT4	AK3
—	—	161	138	32,625
TT	—	8,578	36,853	33,499
Anti-Ig, TT	—	979	6,985	33,029
Anti-Ig, TT	TT	8,552	36,491	ND
TT, anti-Ig	—	5,984	28,616	ND
TT	Anti-Ig	8,425	33,890	ND
Expt 2: Treatment of B cells before fixation				
—	—	127	214	21,779
—	TT	148	171	22,532
TT	—	12,373	79,175	21,708
Chloroquine+TT	—	220	962	21,582

Expt 1: A TT-specific B-cell clone (8.5) was washed, resuspended in RPMI/FCS and incubated on ice with native TT (5×10^{-10}M) or with a rabbit anti-human F(ab')$_2$ affinity-purified antibody (300 µg ml^{-1}). After 15 min, anti-F(ab')$_2$ or anti-TT were added as indicated for 1 h. The cells were then washed three times, irradiated (3,000 R) and cultured with TT-specific T cells as described in Fig 1 legend. Native TT (5×10^{-10} M) or anti-immunoglobulin (300 µg ml^{-1}) were added where indicated. Expt 2: A TT-specific B-cell clone (11.3) was washed, resuspended in RPMI 10% FCS and pulsed at 37 °C with 10^{-9} M native TT or medium alone in the presence or absence of 10^{-5} M chloroquine. After 4 h the cells were washed four times with Hank's balanced salt solution (HBSS) and fixed with 0.05% glutaraldehyde for 1 min at room temperature. The reaction was stopped with 0.2 M glycine in HBSS and the cells were washed three times. Glutaraldehyde-fixed and fresh B cells bound, respectively, 44,331 and 31,520 c.p.m. of ^{125}I-TT per 10^6 cells. Fixed cells (5×10^4) were cultured in 96-well U-bottom microplates with 2×10^4 T cells from TT-specific clones (KT2 and KT4) or an alloreactive clone (AK3) specific for K.K. (the TT-immune donor) as a control. Soluble TT was added in culture at 10^{-7} M where indicated. Thymidine incorporation was measured as in Fig. 1. Data represent the geometric mean of triplicate determinations

As a specificity control, we compared the ability of TT-specific and nonspecific B cells to present two antigens not recognized by their specific surface antibodies. The first was the denatured form of TT itself. As generally found for complex protein antigens[8], denaturation of TT followed by reduction and carboxymethylation results in an antigen preparation that has lost the

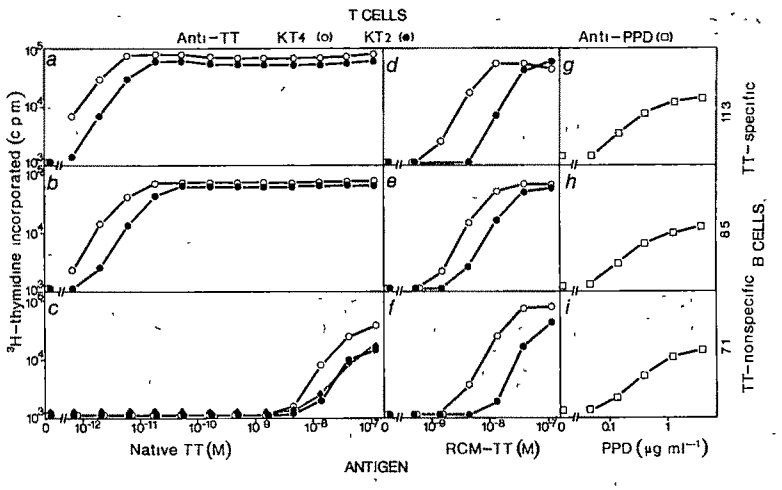


Fig. 1 Antigen presentation by antigen-specific B lymphocytes. Cells (2×10^4) from the TT-specific T-cell clones KT2 (●) and KT4 (○) or from a PPD-specific T-cell line (□) were cultured with 2×10^4 irradiated (3,000 R), EBV-transformed B-cell clones specific for TT (*a, d, g,* clone 11.3; *b, e, h,* clone 8.5) or non-TT-specific (*c, f, i,* clone 7.1) in 200 μl RPMI/FCS in 96-well flat-bottom microplates. The cultures were stimulated with a wide range of concentrations of native TT, reduced carboxymethylated-TT (RCM-TT) or PPD. In *c*, clone KT2 was also stimulated in the presence (◆) of 3 μg ml^{-1} purified antibody produced by clone 11.3. After 2 days the cultures were pulsed for 16 h with 1 μCi ^{3}H-thymidine and the incorporated radioactivity was counted. The background of irradiated B cells alone (always <3,000 c.p.m.) was subtracted.

Methods. Antigens: Native TT was obtained from the Massachusetts Public Health Biological Laboratories and purified by gel filtration on Sephadex G-200. RCM-TT was prepared by denaturation of native TT in 6 M urea, followed by reduction with dithiothreitol and carboxymethylation with iodoacetic acid. The anti-TT antibody produced by specific B cells (see below) was purified from the culture supernatant and coupled to CNBr-activated Sepharose. This immunoadsorbent was used to deplete RCM-TT of contaminating antigen still reactive with these antibodies. This antigen preparation contained <0.1% antigen still reactive with antibody as detected by inhibition of binding of ^{125}I-TT. PPD was obtained from Serum Institut, Copenhagen. T cells: T-cell clones specific for TT were derived as described previously[11]. Briefly, peripheral blood mononuclear cells (PBM) from a TT-immune donor (K.K.) were stimulated *in vitro* with 10^{-7} M TT. After 7 days, the activated T cells were expanded in medium supplemented with 30 U ml^{-1} recombinant interleukin-2 (IL-2, Biogen) and 2 weeks later restimulated with TT and autologous irradiated (3,000 R) PBM. After a further expansion in IL-2, the cells were cloned by limiting dilution in the presence of irradiated autologous PBM, TT and IL-2. Five TT-specific clones were isolated and maintained in culture for more than 8 months by periodic restimulations with antigen, followed by expansion in IL-2. All the T cells used in the present experiments were obtained from 3–4-week-old IL-2-dependent cultures and were virtually devoid of contaminating APC, as they were unable to proliferate in response to soluble antigen unless appropriate APC were added. PPD-specific T cells from donor K.K. were also obtained from PBM stimulated with PPD. The cells were selected by three cycles of restimulation and grown in IL-2 as described above. B cells: TT-specific B-cell clones were obtained from donor K.K. by EBV transformation of peripheral blood B cells essentially as described elsewhere[13]. PBM (10^7) were resuspended in 10 ml RPMI/FCS containing 30% supernatant of the EBV-producing marmoset cell line B95.8 and 600 ng ml^{-1} cyclosporin A (Sandoz). After 16 h at 37 °C, the cells were washed and seeded at 5×10^4 cells per well in 96-well flat-bottom microplates in 200 μl RPMI/FCS containing 600 ng ml^{-1} cyclosporin A. After 13 days the supernatants were tested for the presence of IgG anti-TT antibodies by enzyme-linked immunosorbent assay[11]. Cells from eight positive wells were cloned and subcloned by limiting dilution in the presence of 3×10^5 rat thymocytes as filler cells. Three different TT-specific clones producing IgG anti-TT antibody (0.5–5 μg ml^{-1}) were isolated as well as several other clones producing IgG not specific for TT.

ability to react with antibodies while maintaining the capacity to stimulate T cells. Figure 1*d–f* shows that there is no significant difference in the low efficiency with which TT-specific and nonspecific B cells present the denatured form of TT. As a further control, an unrelated antigen, purified protein derivative of tuberculin (PPD), was used. No difference was found between TT-specific and nonspecific B cells in their capacity to present PPD to a PPD-specific T-cell line (Fig. 1*g–i*).

To test the possibility that secreted antibodies contribute to the high efficiency of antigen presentation by specific B cells, monoclonal anti-TT IgG was purified from the culture supernatants of the B-cell clones and added at different concentrations to cultures containing TT-specific T-cell clones and nonspecific B cells. In no case was any significant increase in the efficiency of presentation of TT observed (Fig. 1*c*). From the above experiments, we conclude that surface immunoglobulin molecules have a role in antigen uptake and concentration on specific B cells, allowing them to cooperate preferentially with specific T cells in the presence of minute amounts of antigen.

To investigate whether specific T/B-cell cooperation, like antigen presentation by macrophages or nonspecific B cells[3,5], is MHC-restricted, TT-specific B cells were compared for their ability to present TT to autologous or allogeneic TT-specific T-cell clones. TT-specific B cells were unable to present TT to allogeneic T cells even in the presence of concentrations of antigen 10^4 times higher than those required for presentation to autologous T cells (Fig. 2*a*). Furthermore, monoclonal anti-HLA-DR antibody quantitatively inhibited antigen presentation (Fig. 2*b*). We conclude, therefore, that these antigen-specific T/B-cell interactions are MHC-restricted.

Macrophages and nonspecific B cells must usually first internalize and process a complex protein antigen before presenting it to T cells[4,6]. However, it was possible that specific B cells could bypass this step and present protein antigens bound externally to immunoglobulin receptors. We therefore tested the effect of anti-immunoglobulin antibodies on antigen uptake and presentation by specific B cells. The addition of anti-immunoglobulin to B cells before pulsing with limiting concentrations of TT effectively reduced their subsequent capacity to stimulate T cells (Table 2, expt 1), the same treatment resulting in an 80% inhibition of uptake of ^{125}I-TT (data not shown). However, when anti-immunoglobulin was added after TT, no inhibition was observed, indicating that these antibodies interfere only with the uptake but not with the subsequent antigen presentation to T cells. We next tested the effect of chloroquine treatment, which inhibits intracellular antigen processing[9], followed by glutaraldehyde fixation to prevent any further processing[10]. Table 2 (expt 2) shows that glutaraldehyde-fixed TT-specific B cells were able to present native TT to TT-specific T cells only if the B cells had been pulsed with TT before fixation. Unpulsed glutaraldehyde-fixed B cells did not present soluble TT, even though they could still bind ^{125}I-TT. If chloroquine was added during the antigen pulse, the ability of TT-pulsed B cells to stimulate T cells was completely abrogated. None of the above treatments, however, affected the capacity of B cells to present an alloantigen to a specific alloreactive T-cell clone. These data indicate that a chloroquine-sensitive intracellular step is required for antigen presentation and that the role of surface immunoglobulin is limited to antigen uptake.

Our finding that specific B cells can present TT to T cells at free antigen concentration ~10^4 times lower than required for presentation by nonspecific B cells or peripheral blood monocytes[11] agree with results reported by Rock *et al.* using a hapten–carrier conjugate and hapten-specific normal B cells[7]. These experiments provide direct quantitative evidence for the role of surface immunoglobulin in concentrating antigen on specific B cells and suggest that this may be the basis of clonal selection in the specific antibody response to low antigen concentrations. Furthermore, our data obtained with a monovalent protein antigen show that selective interaction can occur at antigen concentrations far lower than the affinity constant of the antibody, that is, in conditions where only a very small

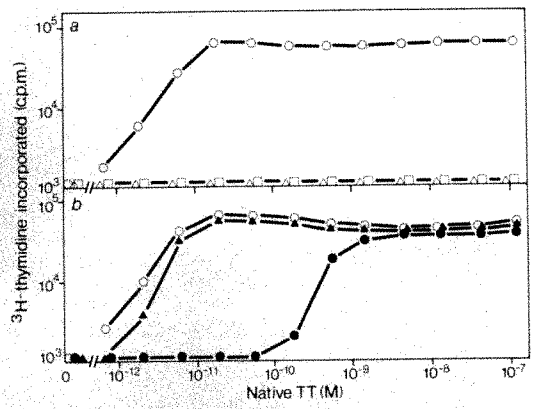


Fig. 2 Antigen presentation by antigen-specific B cells is MHC-restricted. *a*, A TT-specific B-cell clone (11.3) was irradiated and cultured in the presence of different concentrations of TT with an autologous TT-specific T-cell clone, KT1 (○), or with allogeneic TT-specific clones AT1 (□) and FT4 (△). The latter clones, obtained as described in Fig. 1 legend from two donors unrelated to K.K., incorporated in parallel experiments >30,000 c.p.m. when stimulated by TT in the presence of autologous APC (data not shown). *b*, TT-specific T and B cells (clones KT4 and 11.3) were stimulated with TT in the presence of anti-HLA-DR monoclonal antibody D1.12 at 10 μg ml^{-1} (●), anti-HLA-A,B,C antibody W6/32 at 50 μg ml^{-1} (▲) or medium alone (○). Culture conditions were as described in Fig. 1 legend.

fraction of surface immunoglobulin is occupied by antigen. It is possible that receptor internalization, by continuously removing antigen from the equilibrium, could lead with time to selective antigen accumulation in specific B cells.

The above experiments assayed T/B-cell interaction by measuring T-cell activation. We also have evidence that B-cell responses, such as increased antibody production and induction of terminal B-cell differentiation, can occur in the same system at the same antigen dose range. Thus, despite the limitations inherent in the use of EBV-transformed B cells and long-term T-cell clones, we feel that these data on antigen presentation are representative of T/B-cell cooperative interaction.

The requirement for antigen processing by specific B cells allows, in principle, all T cells selected by macrophage-processed antigen to interact with the same specificity with all antigen-specific B lymphocytes, independently of the hapten epitope recognized by the B cell. The requirement for processing may vary with the form of antigen used for stimulation[10], however, since the B-cell response is focused mainly against conformational determinants[8], which are easily lost on processing, it is likely that antigen processing by B cells will be a quite general rule. Antigen processing by B cells also easily explains the linked cooperation between two cells of different specificities. B cells bind soluble antigens by recognizing easily accessible conformational epitopes and, after intracellular processing of the antigen, will present to the T cells a different series of determinants similar to those produced by any nonspecific APC. This suggests that the antigen bridge is due to a sequential, rather than simultaneous, recognition of antigen by B and T cells.

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1. Mitchison, N. A. *Eur. J. Immun.* **1**, 18-27 (1971).
2. Rajewsky, K., Schirrmacher, W., Nase, S. & Jerne, N. K. *J. exp. Med.* **129**, 1131-1136 (1969).
3. Rosenthal, A. S. & Shevach, E. M. *J. exp. Med.* **138**, 1194-1212 (1973).
4. Unanue, E. R., Beller, D. I., Lu, C.Y. & Allen, P. M. *J. Immun.* **132**, 1-5 (1984).
5. Katz, D. H., Hamaoka, M. E. & Benacerraf, B. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2624-2628 (1973).
6. Kakiuki, T., Chestnut, R. W. & Grey, H. M. *J. Immun.* **131**, 109-114 (1982).
7. Rock, K. L., Benacerraf, B. & Abbas, A. K. *J. exp. Med.* **160**, 1102-1113 (1984).
8. Benacerraf, B. *J. Immun.* **120**, 1809-1812 (1978).
9. Ziegler, H. K. & Unanue, E. R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 175-178 (1982).
10. Shimonkevitz, R., Kappler, J., Marrack, P. & Grey, H. *J. exp. Med.* **158**, 303-316 (1983).
11. Lanzavecchia, A., Parodi, B. & Celada, F. *Eur. J. Immun.* **13**, 733-738 (1983).
12. Celada, F., Macario, A. J. L. & Conway De Macario, E. *Immunonochemistry* **10**, 797-804 (1973).
13. Rosen, A., Persson, K. & Klein, G. *J. Immun.* **130**, 2899-2902 (1983).

Absence of growth by most receptor-expressing fetal thymocytes in the presence of interleukin-2

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The growth of mature T cells is regulated by receptors for interleukin-2 (IL-2) and by IL-2 itself. Binding of antigen to T-cell antigen receptors induces the expression of IL-2 receptors, and binding of IL-2 to these receptors induces transferrin receptor expression and is sufficient to promote the growth of T cells for several days[1,2]. However, nothing is known about the growth requirements of pre-T cells. We have therefore studied the dividing population of T-cell precursors which carry the Thy-1 surface antigen, but lack surface antigens Ly2 and L3T4; these cells are present in 14-day-old embryonic thymus[3,4]. If the thymus is removed at this stage and placed in organ culture, all lymphocyte subpopulations normally present in thymuses of adult mice develop *in vitro*[4,5], that is, the nonfunctional Ly2$^+$, L3T4$^+$ population and the functional Ly2$^+$, L3T4$^-$ and Ly2$^-$, L3T4$^+$ populations[4]. We now report that, in contrast to their progeny, the early Ly2$^-$, L3T4$^-$ cells express large amounts of IL-2 receptors, but most of them do not grow in IL-2-containing media outside the thymus. In contrast to dividing mature T cells, most fetal thymocytes express low amounts of transferrin receptors.

We have analysed the expression of receptors for IL-2 and transferrin using monoclonal antibody 7D4, directed against IL-2 receptors[6], and antibody 34D2, directed against transferrin receptors. The 34D2 hybridoma was obtained in our laboratory, and the antibodies precipitate a disulphide-linked dimer, consisting of subunits of relative molecular mass 93,000 (93K) and inhibit the binding of radiolabelled transferrin to the EL4 thymoma (A.C., unpublished). Table 1 shows the proportion of IL-2-receptor-positive thymocytes at various stages of thymus development; >90% of 14-day-old embryonic thymocytes were IL-2-receptor positive whereas only about 5% of adult thymocytes were stained. Moreover, 15-day-old embryonic thymocytes which showed very strong staining with antibody 7D4, stained only weakly with the anti-transferrin receptor antibody 34D2 in contrast to cells from the IL-2-dependent line CTL-L16 (Fig. 1). As analysed by gel electrophoresis, antibody 7D4 precipitated the same molecular species from both the fetal thy-

Table 1 The proportion of IL-2-receptor-positive thymocytes at various stages of thymus development

Age	7D4 positive
14-Day embryonic	90%
15-Day embryonic	72%
16-Day embryonic	20%
21-Day postnatal	4%

Thymocytes from various donors were incubated with 7D4 antibodies, washed and stained with a fluorescein isothiocyanate (FITC)-labelled sheep anti-mouse immunoglobulin (kindly provided by Luciana Forni) which cross-reacts on rat antibodies. Stained cells were analysed by fluorescence-activated cell sorter (FACS) as described elsewhere[4] and shown in Fig. 1.

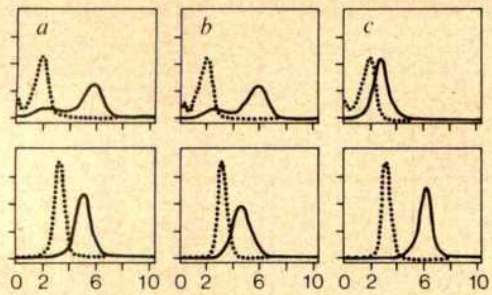


Fig. 1 Staining of 15-day-old embryonic thymocytes (top) and CTL-L16 cells (bottom) with anti-IL-2-receptor antibody 7D4 (hybridoma supernatant undiluted (*a*) and diluted 1:10 (*b*)) and anti-transferrin-receptor antibody 34D2 (undiluted supernatant (*c*)). Cells were incubated with antibodies at 0 °C, washed and stained with FITC-sheep anti-mouse immunoglobulin. Broken line, second antibody only.

mocytes and the CTL-L16 line (Fig. 2). We did not detect any IL-2 production by 15-day-old embryonic thymocytes[4] and, despite their expression of IL-2 receptors, fetal thymocytes did not grow outside the thymus in IL-2-containing media (Table 2).

The biological significance of IL-2 receptor expression by pre-T cells is obscure. As a first step towards an understanding, we may compare the results presented here with the data available on the induction and function of IL-2 receptors in mature T cells. First, IL-2 receptor expression by mature T cells is controlled by antigen receptors which are, at least in part, formed by two different ~45K polypeptide chains linked by disulphide bridges[7,8]. Although 15-day-old embryonic thymocytes do not express these antigen receptor molecules, which are detectable in two-dimensional gel analysis[9], they nevertheless express high levels of IL-2 receptors (Fig. 1). At present, however, we cannot exclude the possibility that only one of the α, β or γ polypeptide chains controls IL-2 receptor expression in fetal thymocytes. Second, IL-2, which induces growth in IL-2-receptor-expressing mature T cells, does not induce DNA synthesis in IL-2-receptor-expressing pre-T cells. Third, proliferating mature T cells express more transferrin receptors than do most fetal thymocytes. The low level of transferrin receptor expression by fetal thymocytes may be due to the lack of IL-2, which is required for transferrin receptor expression by mature T cells. The expression of IL-2 receptors in the absence of IL-2 may, in fact, be a signal for cell death and related to the finding that most newly formed thymocytes die *in situ*[10].

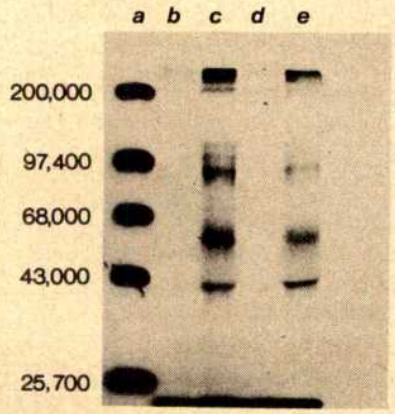


Fig. 2 Precipitation of the antibody 7D4 from lysates of CTL-L16 cells and 15-day-old embryonic thymocytes (pre-T cells). SDS-polyacrylamide gel electrophoresis (10%, reducing conditions) of material precipitated by antibody 7D4. The CTL-L16 line as well as 15-day-old embryonic thymocytes were labelled with ^{125}I using lactoperoxidase. NP40 lysates were precipitated with 7D4 or normal rat serum and rabbit anti-rat immunoglobulin. Lane *a*, relative molecular mass markers; lane *b*, CTL-L16 and rat serum; lane *c*, CTL-L16+7D4; lane *d*, pre-T cells+rat serum; lane *e*, pre-T cells+7D4.

Table 2 ^{3}H-thymidine incorporation by fetal thymocytes and CTL-L16

IL-2	15-Day-old embryonic thymocytes	CTL-L16
—	6,700	9,200
1,000 U ml^{-1}	6,900	115,400

Cells (10^5) were suspended in culture medium with or without IL-2 and ^{3}H-thymidine was added immediately. Incorporation of thymidine was measured after 24 h. After 48 h no viable thymocytes were detected. Recombinant IL-2 was obtained from Biogen[11].

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1. Larsson, E. L. & Coutinho, A. *Nature* **280**, 239-241 (1979).
2. Devos, R., Plaetinck, G. & Fiers, W. *Eur. J. Immun.* (in the press).
3. Jenkinson, E. J., Owen, J. J. T. & Aspinall, R. *Nature* **284**, 177-179 (1980).
4. Kisielow, P., Leiserson, W. & von Boehmer, H. *J. Immun.* **133**, 1117-1123 (1984).
5. Robinson, J. M. & Owen, J. J. T. *Clin. exp. Immun.* **27**, 322 (1977).
6. Malek, T. R., Robb, R. J. & Shevach, E. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5694-5698 (1983).
7. Haskins, K. *et al. J. exp. Med.* **157**, 1149-1169 (1983).
8. Acuto, O., Meuer, S. C., Schlossman, J. C. & Reinherz, E. L. *J. exp. Med.* **158**, 1368-1373 (1983).
9. Snodgrass, H. R., Kisielow, P., Kiefer, M., Steinmetz, M. & von Boehmer, H. *Nature* **313**, 592-595 (1985).
10. McPhee, D., Pye, J. & Shortman, K. *Thymus* **1**, 151-156 (1979).
11. Devos, R. *et al. Nucleic Acids Res.* **11**, 4307-4323 (1983).

Sequence homology of the LFA-1 and Mac-1 leukocyte adhesion glycoproteins and unexpected relation to leukocyte interferon

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Cell-surface adherence reactions are fundamental to the biology of lymphocytes, monocytes and granulocytes. The lymphocyte function-associated 1 (LFA-1) and macrophage 1 (Mac-1) glycoproteins mediate differing types of adhesion reactions on these cells. LFA-1 participates in T-lymphocyte and natural killer-cell adhesion to target cells[1-3], whereas the Mac-1 antigen is identical to the complement receptor type 3, which mediates adhesion of monocytes and granulocytes to C3bi-sensitized particles[4]. Deficiency of these proteins, in a heritable disease, results in multiple adhesion-related leukocyte defects[5,6]. LFA-1 and Mac-1 resemble one another in overall structure, having α-subunits of relative molecular mass (M_r) 180,000 and 170,000, respectively, which are non-covalently associated with β-subunits of M_r 95,000 in $\alpha_1\beta_1$ complexes[7,8]. Peptide mapping and immunological cross-reactivity have shown that the β-subunits are highly related if not identical, but have revealed no similarities between the α-subunits[7,9,10]. Nonetheless, the shared β-subunit suggested that LFA-1 and Mac-1 might be members of a protein family containing diversified but evolutionarily related α-subunits. Therefore, we examine here the structure of the α-subunits by N-terminal amino-acid sequencing. Sequence homology shows that the α-subunits are members of a novel leukocyte adhesion protein family, and suggests that their evolution occurred by gene duplication. A search for similarities to previously sequenced proteins reveals a further unexpected homology between LFA-1 and leukocyte (α) interferons.

```
                1                  10                  19
LFA-1   (Tyr)Asn Leu Asp Thr Arg Pro Thr Gln Ser Phe Leu Ala Gln Ala(Gly)Arg(His)Phe
Mac-1   (Phe)Asn Leu Asp Thr Glu His(Pro)Met Thr Phe Glx Glu Asn Ala Lys(Gly)Phe
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Fig. 1 N-terminal sequences of LFA-1 and Mac-1 α-subunits. Homologous sequences are boxed.

Methods. LFA-1 was purified from 10- or 50-g batches of EL-4 T lymphoma cells grown *in vitro*. Cells were lysed with Triton X-100, sodium deoxycholate was added before the ultracentrifugation step and LFA-1 antigen was bound to M17/4 monoclonal antibody-Sepharose and eluted at high *p*H, as described previously[8]. The eluate in 0.5% Triton X-100, 20 mM triethylamine *p*H 11.0, was neutralized by adding 0.075 vol. of 1 M Tris-HCl *p*H 6.8. Mac-1 from P388D$_1$ cells was purified similarly on M1/70 monoclonal antibody-Sepharose, except that the neutralized eluate was subjected to a second cycle of affinity purification. SDS-polyacrylamide gel electrophoresis (PAGE) and Coomassie brilliant blue staining of purified LFA-1 and Mac-1 demonstrated that the α- and β-subunits had no contaminants, as shown previously[7,10]. Purified antigens were reduced with 20 mM diothiothreitol, alkylated with 50 mM iodoacetic acid and precipitated overnight at −20 °C with 1.5 vols 1% acetic acid in methanol. Precipitates were collected at 200,000 *g* for 0.5 h and subjected to preparative SDS-PAGE. Subunits were localized by Coomassie brilliant blue staining, electroeluted[17] and subjected to gas/liquid solid phase microsequencing[18]. Phenylthiohydantoin amino acids were determined by HPLC[19]. The sequence assignments shown were confirmed by multiple determinations. The α-subunit of LFA-1 from two different purifications was sequenced three times (200, 44 and 22 μg) and the α-subunit of Mac-1 was sequenced twice (63 and 31 μg). Residues in parentheses were found together with smaller amounts of another residue, or in low yield. No sequence was obtained from the LFA-1 and Mac-1 β-subunits, suggesting that they are blocked.

We purified murine LFA-1 and Mac-1 antigens by monoclonal antibody affinity chromatography from detergent lysates of EL-4 T-lymphoid and P388D$_1$ macrophage-like cell lines and separated the α-and β-subunits (see Fig. 1 legend). The N-terminal sequences of the LFA-1 and Mac-1 α-subunits are 33% homologous (Fig. 1). Four contiguous amino acids are identical at positions 2–5 and further identities occur at positions 11 and 15. Some of the differences represent relatively conservative amino-acid substitutions, namely Phe for Tyr at residue 1, Thr for Ser at 10, and Asp for Glu at 14. If a gap was introduced before the Lys at position 16 in Mac-1, it would be aligned with an Arg in LFA-1 and Phe at positions 18 and 19 would be aligned.

The sequence homology shows that the α-subunits constitute a novel adhesion protein family, the genes of which evolved by duplication of a primordial ancestral gene. The N-terminal sequences have diverged considerably since the primordial gene duplication event. These differences suggest that the specificity in adhesive interactions is determined by the α-subunits, in agreement with localization of a binding site of the C3bi antigen on the α-subunit of Mac-1 using α- and β-subunit-specific monoclonal antibodies[10,11]. Duplication and specialization of the α-subunits must have preceded divergence of mouse and human, as LFA-1 and Mac-1 homologues mediating the same functions occur in humans[12]. A third type of human α-subunit has been identified[12] which may represent a further member of the α-subunit protein family. The amino-acid sequences described here allow the specification of oligonucleotide probes, which should be of use in the isolation and further study of the Mac-1/LFA-1 family of α-subunit genes.

Table 1 Homology between LFA-1, Mac-1 and interferons

	% Identity				
	Mac-1	Rodent IFN-α	Human IFN-α	IFN-β	IFN-γ
LFA-1	33	32	35	26	5
Mac-1	—	15	7	3	6
Rodent IFN-α	—	—	55	20	0
Human IFN-α	—	—	—	27	1
Murine and human	—	—	—	—	5

The numbers refer to the percentage homology between the sequences depicted in Figs 1 and 2. Homologies between residues 7–25 of the mouse and human interferons were calculated and averaged in the groups shown.

A computer search[13] for homologies with other proteins reveals an unexpected, strong homology between LFA-1 and human leukocyte interferon (Fig. 2). Multiple genes for α-interferon (IFN-α) code for a family of closely related interferon proteins[14]. Comparison of LFA-1 with 12 mouse, rat and human α-interferons reveals homology of LFA-1 with all of them (Fig. 2). Note that the LFA-1 and IFN-α sequences align without any gaps. IFN-α shows ~30% homology to fibroblast interferon (IFN-β). LFA-1 shows a lesser degree of homology with IFN-β (Fig. 2). The homologies with IFN-α are highly statistically significant (Fig. 2).

Homology with LFA-1 occurs in different positions in rodent and human IFN-αs. Despite the higher homology of LFA-1 to human IFN-α1 than to any other interferon, an additional homology found exclusively with rodent IFN-α occurs at residues 1 and 2 in LFA-1 (Fig. 2). Also, conserved substitutions occur at many positions, including 10 and 11, where no identities are found. These findings further strengthen the homology between LFA-1 and IFN-α.

The homology among LFA-1, Mac-1 and the interferons is summarized in Table 1. Mac-1 and LFA-1 are about as homologous with one another as is LFA-1 with IFN-α. The significance of the 32–35% homology between LFA-1 and IFN-αs is emphasized by the fact that it is stronger than the 20–27% homology observed between IFN-α and IFN-β in the segments compared with LFA-1, or the homology of 29% along the total lengths of IFN-α and IFN-β[15]. Furthermore, the homology between LFA-1 and the α- and β-interferons is stronger than the homology between IFN-γ and the α- and β-interferons.

Our findings suggest that the LFA-1/Mac-1 α-subunit family and the interferon family arose from a single ancestral gene and can be considered as a superfamily. This is remarkable for

```
                                1                   5                  10                  15                  19   P(%)b
LFA-1           (1-19)  Tyr Asn Leu Asp Thr Arg Pro Thr Gln Ser Phe Leu Ala Gln Ala Gly Arg His Phe          -
Mouse IFN-α1    (7-25)  His Asn Leu Arg Asn Lys Arg Ala Leu Thr Leu Leu Val Gln Met Arg Arg Leu Ser          1.2
Mouse IFN-α2    (7-25)  Tyr Asn Leu Arg Asn Lys Arg Ala Leu Lys Val Leu Ala Gln Met Arg Arg Leu Pro          0.3
Rat   IFN-α     (7-25)  His Asn Leu Arg Asn Lys Arg Val Phe Thr Leu Leu Ala Gln Met Arg Arg Leu Ser          2.0
Human IFN-α1    (7-25)  His Ser Leu Asp Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser          0.08
Human IFN-α2    (7-25)  His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Lys Ile Ser          0.6
Human IFN-α4    (7-25)  His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser          0.08
Human IFN-α5    (7-25)  His Ser Leu Ser Asn Arg Arg Thr Leu Met Ile Met Ala Gln Met Gly Arg Ile Ser          0.16
Human IFN-α6    (7-25)  His Ser Leu Gly His Arg Arg Thr Met Met Leu Leu Ala Gln Met Arg Arg Ile Ser          0.4
Human IFN-α7    (7-25)  His Ser Leu Arg Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser          0.13
Human IFN-α8    (7-25)  His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Arg Arg Ile Ser          0.5
Human IFN-αH    (7-25)  His Ser Leu Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser          0.5
Human IFN-αC    (7-25)  His Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser          0.1
Mouse IFN-β     (10-29) Glu Arg Thr Asn Ile Arg Lys Cys Gln Glu Leu Leu Glu Gln Leu Gly Lys Ile Asn          3.2
Human IFN-β     (10-29) Gln Arg Ser Ser Asn Phe Gln Cys Gln Lys Leu Leu Trp Gln Leu Gly Arg Leu Glu          126
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Fig. 2 Sequence homology between LFA-1 and interferons. Residues homologous between interferons and LFA-1 are boxed; homologies with Mac-1 are italicized. α-, β- and γ-interferon sequences[14,20,21] were aligned as described previously[22,23]. Asp 25 of IFN-β was deleted for alignment[22]. The right-hand column describes the percentage probability that homology results from chance alone, as determined by summation of the log odds of each amino-acid pair derived from the mutation data matrix of Dayhoff[24].

several reasons. First, LFA-1 and Mac-1 are surface receptors, whereas the interferons are secreted mediators. Second, there are striking differences in size. The LFA-1 and Mac-1 α-polypeptide chains (M_r 180,000 and 170,000) are roughly 10 times longer than those of the interferons ($M_r \sim 20{,}000$). Despite the difference in size, the N-terminal sequence of LFA-1 aligns only six residues away from the N-terminus of IFN-α. A common evolutionary mechanism for obtaining large proteins is tandem duplication and joining of domains. Further sequencing of LFA-1 is needed to determine whether it contains the homologue of an entire interferon domain and whether it contains duplications of these domains.

It is tempting to speculate that the LFA-1/Mac-1/interferon superfamily could have functional as well as structural interrelationships. All of these molecules function in cell interactions of the host defence system. Unlike the immunoglobulin superfamily[16], none of them is involved in specific antigen recognition and their importance in host defence could have pre-dated the evolution of specific immunity.

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1. Davignon, D., Martz, E., Reynolds, T., Kürzinger, K. & Springer, T. A. *J. Immun.* **127**, 590-595 (1981).
2. Springer, T. A., *et al. Immun. Rev.* **68**, 111-135 (1982).
3. Pierres, M., Goridis, C. & Golstein, P. *Eur. J. Immun.* **12**, 60-69 (1982).
4. Beller, D. I., Springer, T. A. & Schreiber, R. D. *J. exp. Med.* **156**, 1000-1009 (1982).
5. Anderson, D. C. *et al. J. clin. Invest.* **74**, 536-551 (1984).
6. Springer, T. A., Thompson, W. S., Miller, L. J., Schmalstieg, F. C. & Anderson, D. C. *J. exp. Med.* **160**, 1901-1918.
7. Kürzinger, K., Ho, M. K. & Springer, T. A. *Nature* **296**, 668-670 (1982).
8. Kürzinger, F. & Springer, T. A. *J. biol. Chem.* **257**, 12412-12418 (1982).
9. Trowbridge, I. S. & Omary, M. B. *J. exp. Med.* **154**, 1517-1524 (1981).
10. Sanchez-Madrid, F., Simon, P., Thompson, S. & Springer, T. A. *J. exp. Med.* **158**, 586-602 (1983).
11. Wright, S. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 5699-5703 (1983).
12. Sanchez-Madrid, F., Nagy, J., Robbins, E., Simon, P. & Springer, T. A. *J. exp. Med.* **158**, 1785-1803 (1983).
13. Doolittle, R. F. *Science* **214**, 149-159 (1981).
14. Weissmann, C. *et al. Phil. Trans. R. Soc.* B**299**, 7-28 (1982).
15. Taniguchi, T. *et al. Nature* **285**, 547-549 (1980).
16. Williams, A. F. *Nature* **308**, 12-13 (1984).
17. Hunkapiller, M. W., Lujan, E., Ostrander, F. & Hood, L. E. *Meth. Enzym.* **91**, 227-236 (1983).
18. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. *J. biol. Chem.* **256**, 7990-7997 (1981).
19. Hunkapiller, M. W. & Hood, L. E. *Meth. Enzym.* **91**, 486-493 (1983).
20. Higashi, Y. *J. biol. Chem.* **258**, 9522-9529 (1983).
21. Gray, P. W. & Goeddel, D. V. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5842-5846 (1983).
22. Sternberg, M. J. E. & Cohen, F. E. *Int. J. biol. Macromolec.* **4**, 137-144 (1982).
23. Epstein, L. B. *Nature* **295**, 453-454 (1982).
24. Dayhoff, M. O. & Hunt, L. T. *Meth. Enzym.* **91**, 524-545 (1983).

Isolation and characterization of a cDNA clone encoding the murine homologue of the human 20K T3/T-cell receptor glycoprotein

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The antigen receptor on the surface of human T lymphocytes, which consists of a heterodimer of relative molecular mass (M_r) 90,000 (90K) (α- and β-chains), is associated with the T3 antigen ($\gamma = 25$K, $\delta = 20$K and $\varepsilon = 20$K)[1-7]. A working model for the mode of action of the T3/T-cell receptor complex is that the clonotypic α- and β-chains are involved in the recognition and binding of antigen in the context of polymorphic major histocompatibility complex (MHC) gene products on the surface of target cells. Antigen binding by the clonotypic receptor probably results in conformational changes in this structure which are recognized by and subsequently trigger the associated T3 complex to transmit signals into the cell, resulting in a proliferative response[8-10]. The similarity in structure between murine and human clonotypic antigen receptors[11-15] suggests that such a mechanism of recognition and activation also exists in mouse T lymphocytes, but so far there has been no evidence for the existence of a murine T3 complex. Here we demonstrate the existence of a T3δ-chain mRNA in murine T lymphocytes. Our sequence data strongly suggest that this mouse mRNA codes for a complete T3δ polypeptide chain and reveal some interesting properties of the protein.

The cDNA clone pPGBC-9, which contains a full-length cDNA copy coding for the human T3δ-chain[16], was used here to examine a series of murine T-cell lines for expression of RNA coding for the murine equivalent of the T3δ-chain; only in mouse T cells was a 0.7-kilobase (kb) mRNA observed (data not shown). From one of these cells, B3C6, an anti-Ia T-T hybridoma,[17] we isolated poly(A)$^+$ mRNA, which was used to construct a cDNA library in the bacteriophage λgt10 (refs 18, 19). This library was then screened with the cDNA insert of the human T3δ cDNA clone pPGBC-9 at low stringency. Ten plaques out of $\sim 2.5 \times 10^5$ were found to hybridize with pPGBC-9. On plaque purification and rescreening at low stringency, only two plaques remained positive. The cDNA inserts of these two overlapping phages were 200 and 640 base pairs (bp) long, respectively. The 640-bp insert of phage λ PEM-T3δ was isolated and subcloned into the *Eco*RI site of the plasmid vector SP65 (ref. 20) (that is, cDNA clone pPEM-T3δ). The nucleotide sequence of clone pPEM-T3δ was determined by the Maxam-Gilbert method[21]. Figure 1 shows the sequencing strategy and the resulting 570-nucleotide-long sequence of the cDNA insert of pPEM-T3δ. Only one open reading frame is present in this sequence, which ends with a termination codon, TAA, at position 490. No initiation codon was detected. Comparison of the size of the cDNA insert with the size of the T3δ mRNA detected in a Northern blot (data not shown) and comparison with the human T3δ cDNA (Fig. 1*b*) indicates that this cDNA does not represent a full-length transcript of the murine T3δ mRNA. Alignment with the human cDNA sequence (Fig. 1*b*) and the presence of a polyadenylation signal, AATAAA, followed by a poly(A) tail 19 nucleotides downstream, shows that pPEM-T3δ contains the entire 3′ noncoding region and lacks some of the 5′ sequences.

We have shown previously that in human the T3δ mRNA is present in cells of the T lineage only[16]. On Northern blot analysis with the murine clone pPEM-T3δ cDNA, mRNA was detected in T cells and thymocytes, but not in L-cells, liver cells or a B-cell hybridoma (data not shown). This suggests that the murine T3δ-chain also is expressed exclusively in thymus-derived lymphocytes.

The PROTALN and NUCALN (IntelliGenetics, Inc.) programs[22] revealed no homology between the murine T3δ-chain and T-cell receptor structures, immunoglobulin or Thy-1. In addition, a search of the Dayhoff database using the Wilbur-Lipman algorithm[22,23] did not reveal homology with any known protein.

Comparison of the amino-acid sequences of the human and murine T3δ polypeptide chains showed that they are very similar. Alignment of the amino-acid sequences revealed that the murine T3δ cDNA clone (pPEM-T3δ) codes for only part of the leader sequence (Fig. 2). Furthermore, the mature protein has two additional amino acids at its C-terminus (Lys and Ser). The mature murine T3δ-chain therefore consists of 152 amino acids with a deduced M_r of 16,905 and is slightly larger than its human equivalent (M_r 16,719). The domain organization of the murine T3δ-chain, derived from its hydropathicity profile[24], is very similar to that of the human T3δ-chain (Fig. 3). A 79-residue extracellular domain is followed by a transmembrane



Fig. 1 *a*, Partial restriction map of the murine T3δ-chain and strategy for sequencing of clone pPEM-T3δ. The restriction enzymes used were *Eco*RI, *Xba*I, *Hin*fI, *Dde*I and *Taq*I. Open circles represent 5′-labelling; the closed circle represents 3′-labelling for Maxam-Gilbert sequence analysis. *b*, Comparison of the nucleotide sequences of murine and human T3δ-chain. The 570-nucleotide-long sequence of clone pPEM-T3δ (mouse T3δ) is compared with the 700-nucleotide-long sequence of clone pPGBC-9 (human T3δ). For the alignment we used the NUCALN (IntelliGenetics, Inc.) program. Identical nucleotides are indicated by double dots. The ATG initiation codon in the human T3δ sequence, and the codon coding for the first amino acid of the mature protein (Phe) and termination codon of both human and murine T3δ are indicated by arrows.

Methods. The murine B3C6 cDNA library constructed in phage λgt10 was screened with the cDNA insert of clone pPGBC-9. Approximately 2.5×10^5 plaques were generated and plaque lifts were made on nitrocellulose filters as described elsewhere[27]. The filters were prehybridized at 42 °C in 50% deionized formamide, 5×SSC (1×SSC = 0.15 M NaCl, 0.015 M Na_3-citrate), 5× Denhardt's solution (1×Denhardt = 0.02% Ficoll 400, 0.02% bovine serum albumin and 0.02% polyvinylpyrrolidone), 50 mM sodium phosphate (pH = 6.5), 0.1% SDS and 250 μg ml^{-1} of denatured salmon sperm DNA. Hybridization was carried out in the same buffer for 16 h at 42 °C. The human T3δ-chain cDNA insert of clone pPGBC-9 (ref. 16), ^{32}P-labelled by nick-translation (specific activity $2\text{–}5\times10^8$ c.p.m. μg^{-1}), was used as hybridization probe at 5×10^6 c.p.m. ml^{-1}. After hybridization, the filters were washed three times in 250 ml of 2×SSC plus 0.1% SDS at room temperature, and twice in 250 ml of 2×SSC plus 0.1% SDS at 50 °C for 15–20 min each. The filters were air-dried and exposed to Kodak XAR-5 film at −70 °C using an intensifying screen. Positive plaques were purified by one additional cycle of screening. Single plaques were isolated and amplified through *Escherichia coli* KM392, followed by a medium-scale phage preparation as described previously[27]. Phage DNA was prepared according to standard procedures[27], digested with *Eco*RI and the generated insert was isolated from 0.8% agarose slab gel by adsorption to activated glass[28]. Plasmid SP65 (ref. 20) was linearized with *Eco*RI and the ends were treated with calf intestinal phosphatase to make it suitable as cloning vehicle[29]. Vector and cDNA insert were mixed in a 1:2 molar ratio, followed by ligation in the presence of 1 mM ATP and 10 units of T4 ligase for 13–16 h at 16 °C. Part of the ligation mixture was used to transform *E. coli* MC1061 (ref. 30) to ampicillin resistance. Recombinants were isolated, identified and grown in large cultures using standard techniques[27]. Plasmids were purified through CsCl gradients[27]. Purified cDNA insert was digested with *Xba*I, *Hin*fI, *Taq*I and *Dde*I, and the generated ends were labelled either at their 5′ ends with T4 polynucleotide kinase and [γ-^{32}P]ATP or at their 3′ ends with DNA polymerase I (Klenow fragment) and [α-^{32}P]dNTPs. The labelled fragments were separated by electrophoresis in a 5% polyacrylamide gel and the individual fragments were isolated and purified[21]. These fragments were subsequently treated with a buffer containing 30% (v/v) dimethyl sulphoxide and 1 mM EDTA for 3 min at 90 °C and run through a strand-separating gel[21]. Single strands were isolated, purified and subjected to chemical degradation as described by Maxam and Gilbert[21].

segment (residue 80–106) and a cytoplasmic domain (residues 107–152). A high level of amino-acid sequence conservation is apparent both in the transmembrane (70.4%) and intracellular (77.2%) domains of murine and human T3δ-chain. Much less homology was found in the extracellular domains (58.2%).

The locations of the five cysteines (residues 16, 52, 72, 75 and 103) and two attachment sites for Asn-linked oligosaccharides (residues 17 and 53) are conserved (Figs 2, 3). The murine T3δ-chain, however, contains a third site for Asn-linked glycans at residue 34. The existence of this third consensus sequence (Asn 34–Lys 35–Thr36) for *N*-linked glycosylation in the murine T3δ-chain suggests that the mature glycosylated murine T3δ-chain has a higher relative molecular mass than its human equivalent. The attachment of three oligosaccharide moieties to a relatively short extracellular domain (79 residues) might decrease the immunogenicity of the murine T3δ; this could explain why attempts to prepare rat monoclonal reagents specific for murine T3 have been unsuccessful. The amino-acid sequences around Asn 34 are very different from those in the human polypeptide chain. This suggests that murine anti-T3 monoclonal antibodies recognize a determinant which is located between residues 20 and 39 in the human T3δ-chain. As this area of the molecule carries an oligosaccharide in the murine chain, this putative determinant is probably also located on the surface of the human protein. The existence of an easily accessible determinant on the surface of the human molecule would

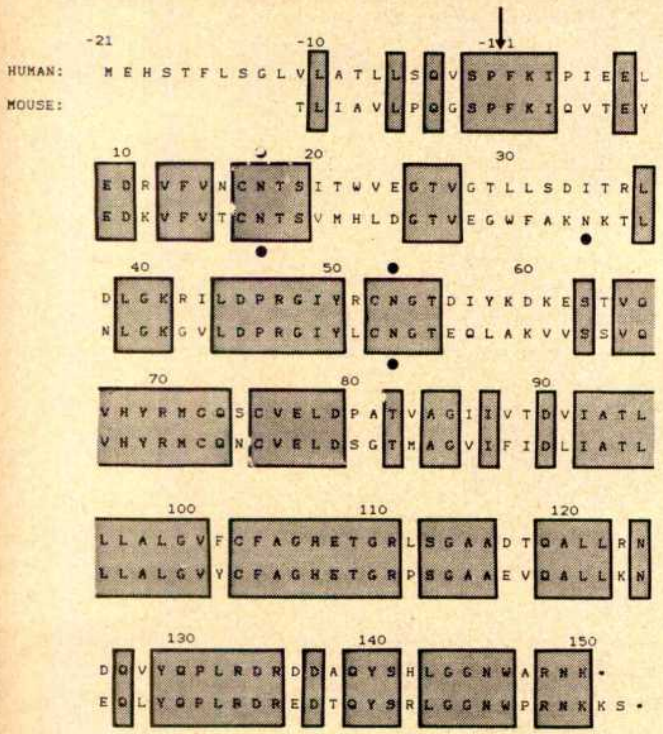


Fig. 2 Comparison of the amino-acid sequences of murine and human T3δ-chain. The vertical arrow above the sequence indicates the first amino acid of the mature protein. Identical amino acids are boxed. The potential attachment sites for asparagine-linked glycosylation are indicated by solid circles.

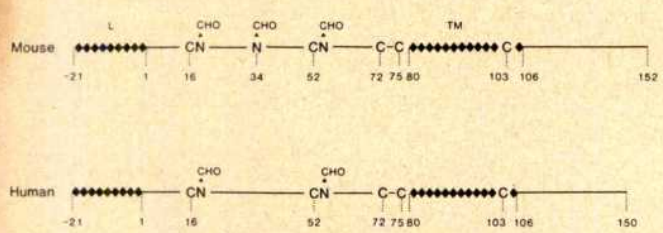


Fig. 3 General features of the organization of the murine and human T3δ-chain. L represents the leader peptide sequence, which is presumably 21 amino acids long in murine T3δ-chain, and TM represents the transmembrane domain. C, cysteine residues; CHO, *N*-linked glycosylation sites.

explain why murine anti-T3 monoclonal antibodies have been prepared with a relatively high frequency.

Of interest is the conservation of the aspartic acid residue at position 90 in the transmembrane domain (Fig. 2). This amino acid could be involved in a salt bridge with the lysine or arginine residues present in the transmembrane segment of the T-cell receptor α- and β-chains (Fig. 4)[14,15,25,26]. The hydrophobic environment may enhance the stabilizing effect of this salt bridge on the protein/protein interactions between the T3δ-chain and the α- and β-chain of the T-cell receptor. Such interaction depends, of course, on the precise positioning of the charged residues.

Thus, we have identified the murine homologue of the T3δ-chain. This finding, together with the similarity in structure of the T-cell receptor α- and β-chains, predicts that murine γ- and ε-chains will be found also. The availability of a cDNA clone coding for the murine T3δ-chain will allow the generation of anti-peptide antibodies, which will be useful in studies of the T3/T-cell receptor complex on the surface of antigen-specific mouse T cells. Furthermore, these antibodies, together with the DNA probes, will allow us to correlate transcriptional activity with cell-surface expression of these cell-surface structures within the thymus. This knowledge should further our understanding of the regulation of the function of the T3/T-cell

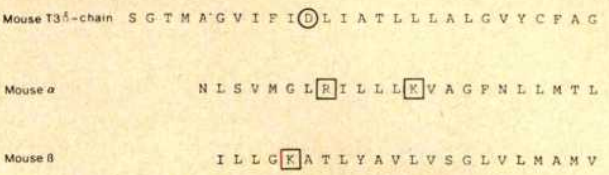


Fig. 4 Amino-acid sequences of the transmembrane regions of murine T3δ-chain and T-cell receptor α- and β-chains aligned at their C-termini. Charged amino-acid residues are indicated.

receptor complex during thymic differentiation and embryonic development.

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1. Borst, J., Prendiville, M-A. & Terhorst, C. *J. Immun.* **128,** 1560–1565 (1982).
2. Borst, J., Alexander, S., Elder, J. & Terhorst, C. *J. biol. Chem.* **258,** 5135–5141 (1983).
3. Borst, J., Prendiville, M. A. & Terhorst, C. *Eur. J. Immun.* **13,** 576–580 (1983).
4. Borst, J. *et al. Nature* **312,** 455–458 (1984).
5. Kanellopoulos, J. M., Wigglesworth, N. M., Owen, M. J. & Crumpton, M. J. *EMBO J.* **2,** 1807–1814 (1983).
6. Meuer, S. C., Acuto, O., Hercend, T., Schlossman, S. F. & Reinherz, E. L. *A. Rev. Immun.* **2,** 23–50 (1984).
7. Oettgen, H. C., Kappler, J., Tax, W. J. M. & Terhorst, C. *J. biol. Chem.* **259,** 12039–12048 (1984).
8. Van Wauwe, J. P., DeMey, J. R. & Goossens, J. G. *J. Immun.* **124,** 2708–2713 (1980).
9. Terhorst, C. *Receptors Recognition* **16,** 131–162 (1984).
10. Oettgen, H. C., Terhorst, C., Cantley, L. C. & Rosoff, P. *Cell* (in the press).
11. Samelson, L. E., Germain, R. N. & Schwartz, R. H. *Proc. natn. Acad. Sci. U.S.A.* **80,** 6972–6976 (1983).
12. Kappler, J. *et al. Cell* **35,** 295–302 (1983).
13. Acuto, O. *et al. J. exp. Med.* **158,** 1368–1373 (1983).
14. Yanagi, Y. *et al. Nature* **308,** 145–148 (1984).
15. Hedrick, S. M., Nielsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308,** 153–157 (1984).
16. van den Elsen, P. *et al. Nature* **312,** 392–397 (1984).
17. Glimcher, L. H. & Shevach, E. M. *J. exp. Med.* **156,** 640–645 (1982).
18. Gubler, U. & Hoffman, B. J. *Gene* **25,** 263–269 (1983).
19. Huynh, T. V., Young, R. A. & Davis, R. W. in *DNA Cloning: A Practical Approach* (ed. Glover, D.) (IRL, Oxford, 1984).
20. Green, M. R., Maniatis, T. & Melton, D. A. *Cell* **32,** 681–694 (1983).
21. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–560 (1980).
22. Wilbur, W. J. & Lipman, D. J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 726–730 (1982).
23. Barker, W. C. *et al. Atlas of Protein Sequence and Structure* (Natn. Biomed. Res. Fdn, Washington, DC, 1984).
24. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157,** 105–132 (1982).
25. Chien, Y. *et al. Nature* **312,** 31–35 (1984).
26. Saito, H. *et al. Nature* **312,** 36–40 (1984).
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
28. Vogelstein, B. & Gillespie, D. *Proc. natn. Acad. Sci. U.S.A.* **76,** 615–619 (1979).
29. van den Elsen, P., Houweling, A. & Van der Eb, A. *Virology* **128,** 377–390 (1983).
30. Casadaban, M. J. & Cohen, S. N. *J. molec. Biol.* **138,** 179–207 (1980).

An unusual type of *V–J* joining diversifies the primary repertoire of mouse λ1 light chains

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It is well established that B lymphocytes can achieve an almost unlimited antibody repertoire by using combinations of at least three different basic mechanisms[1]. First, the mammalian genome has multiple, distinct germline variable (*V*), joining (*J*) and diversity (*D*) gene segments which can presumably combine randomly to give *V–D–J* joins in the heavy (H)-chain loci and *V–J* joins in the light (L)-chain loci during the formation of functional antibody genes. Second, the actual joining points between any two

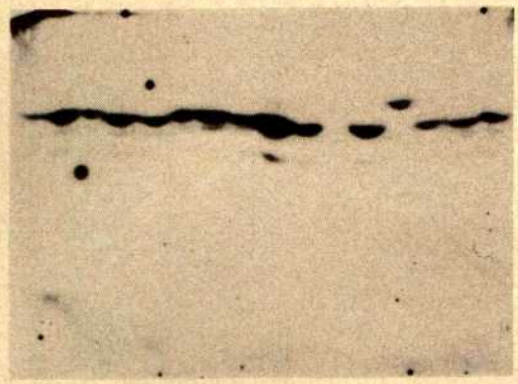


Fig. 1 IEF analysis, which shows one variant λ1 light chain. To obtain the λ1-chain-containing supernatants, normal spleen cells from (BALB/c × NZB)F_1 mice were cultured in the presence of 50 μg ml^{-1} LPS (*Salmonella typhosa* 0901, Difco) in RPMI medium supplemented with 20% fetal bovine serum, 5×10^{-5} M 2-mercaptoethanol and 50 μg ml^{-1} gentamycin. The initial cell concentrations were 5×10^5 ml^{-1} and 3×10^4 ml^{-1} for day 3 and day 8 cultures respectively. Stimulated cells were fused with Sp2-0 plasmacytoma cells using standard procedures[6]. When 10^8 fused cells were plated into 20 96-well microtitre plates, multiple clones grew in individual wells. Cells from those wells that showed a positive reaction with a monoclonal rat anti-mouse λ1 antibody (4/1-101)[3] were subcloned, and the supernatants from these λ1-positive subclones were subjected to IEF analysis. The supernatants were mildly reduced with dithiothreitol (10 mM, 15 min at room temperature) followed by alkylation with 2× molar excess of iodoacetamide (30 min at room temperature). Just before loading, a fourfold volume of IEF sample buffer (9.5 M urea and 2% ampholine *p*H 3.5–10) was added to the supernatants. Usually ~40 μl of solution was applied per lane to 0.8-mm-thick 5% polyacryalamide slab gels (model V-16-2; BRL) containing 9.2 M urea and 2% ampholine *p*H 3.5–10. After overnight focusing at 200 V, the proteins were electrophoretically transferred to nitrocellulose filters using Western blot conditions[7], then the nitrocellulose filters were incubated in blocking solution (1% casein, 50 mM Tris-HCl *p*H 8.0, 150 mM NaCl) for 1 h at room temperature. Finally, λ1 light chains were detected using iodinated anti-λ1 monoclonal antibody, with subsequent autoradiography of the washed filters.

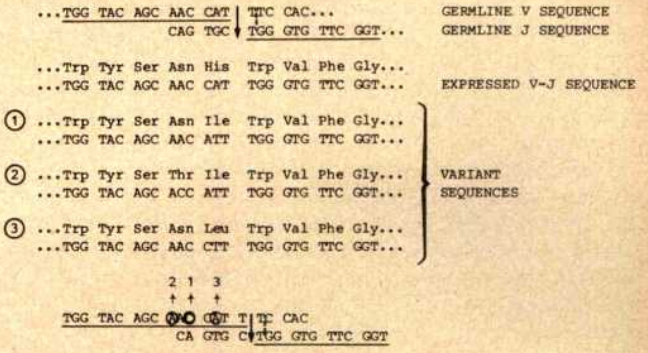


Fig. 2 The *V–J* join most frequently found is shown at the top, with the coding sequences of the *V* and *J* gene segments prior to rearrangement underlined. This 'germline' λ1 join can be compared with those of the variants, whose sequences were determined. All three variants have an extra T residue inserted at the *V–J* junction, and this is compensated for by the deletion of a single residue (circled nucleotides at the bottom) in the adjacent *V* sequence.
Methods. A double-stranded *Sac*I/*Kpn*I restriction fragment (75 base pairs (bp) long) corresponding to the 5′ portion of the λ1 constant-region gene was excised from the complementary DNA clone[8] pABλ1-15 (obtained from Dr A. Bothwell) and used as a primer. Total cytoplasmic RNA (50 ng) and the 5′ end-labelled primer (200 ng) were mixed in a 100-μl volume of annealing buffer (80% dimethylformamide, 400 mM NaCl, 40 mM PIPES *p*H 6.4, 1 mM EDTA) and incubated at 47.5 °C for 90 min. Annealing was stopped by diluting the mixture fourfold with 0.3 M sodium acetate and by precipitating nucleic acids with ethanol. The resultant pellet was dissolved in standard reverse transcription buffer and primer extension carried out using established conditions for reverse transcription[9]. Full-length cDNA was isolated on denaturing 5% polyacrylamide gels, and the sequence of the gel-purified material was determined according to the method of Maxam and Gilbert[10].

combining gene segments can vary considerably, increasing the germline diversity. Further variability is generated in the heavy-chain locus by *de novo* addition of extra nucleotides between the combining gene segments. Finally, somatic mutations independent from joining events can accumulate in *V* regions during the lifetime of B lymphocytes. Here we report that when *V* and *J* regions join in the formation of functional λ1 light-chain genes, 'lethal' out-of-frame joins can be compensated for by the deletion of nucleotides several bases upstream of the actual joining points; this generates small stretches of nucleotides in a new frame between the deletion and the *V–J* joining point, thus creating additional diversity in the third hypervariable regions of the mouse λ1 light chains.

In order to be able to study the mechanisms which generate diversity in the variable regions of mouse antibodies, we decided to minimize the complexity of the experimental system in two ways. First, we concentrated on λ1 light chains because the inherited repertoire of λ1 genes contains only one *V* region whose sequence is known[1]; thus, any different sequences found must result from somatic mutation. Second, we studied only virgin primary B cells and not, for example, immune cells, so that the frequency of variants observed would reflect as closely as possible the true frequency of somatic variation. To do this we stimulated normal spleen cells from (BALB/c × NZB)F_1 mice with bacterial lipopolysaccharide (LPS) which triggers the proliferation of virgin mouse spleen B cells[2]. Stimulated cells were then immortalized by fusion with Sp^2-0 plasmacytoma cells after 3 or 8 days of *in vitro* culture.

We obtained 101 and 113 λ1-secreting clones, as defined by their reaction with rat monoclonal anti-mouse λ1 antibody 4/1-101, (ref. 3), from day 3 and day 8 fusions, respectively. Ninety-five per cent of the day 3 but only 40% of the day 8 clones were IgM producers, showing that our culture conditions, like the limiting dilution cultures described by others[4], allowed the immunoglobulin switch to occur. To detect variants, we used isoelectric focusing (IEF) analysis as a primary screen; although this method underestimates the DNA sequence variability, the IEF method that we developed made it easy to analyse hundreds of supernatants (see Fig. 1 legend). We found four IEF variants among 213 λ1 chains studied (~2%). (Figure 1 shows an example of a primary screen that revealed one variant.) All four variants had similar IEF patterns; these were one charge unit more acidic than the wild-type pattern. The variants seemed to represent a random subpopulation of the whole sample of 213 λ1-secreting clones: two originated from the day 3 fusion, being of IgM type, and of the two day 8 clones, one had switched to IgG.

We determined the DNA sequences of three of the variants and of one wild type by using the primer extension technique (see Fig. 2 legend). It was immediately apparent from the sequence data that the variants were the products of a novel kind of joining mode. All of them had an extra T nucleotide at the *V–J* junction compared with the most frequent *V–J* join (Fig. 1). To maintain the correct reading frame, a compensatory deletion of one base had occurred 2, 4 and 6 nucleotides upstream of the 'inserted' T residue; as a result, only a small stretch of nucleotides was out-of-frame between the deletion and the insertion. In the most extreme case (variant 2) the DNA sequence encodes two new amino acids, Thr-Ile, at positions 95 and 96 compared with the wild-type sequence (Asn-His). The switch from the basic amino acid His to the neutral Ile or Leu (His is positively charged at the isoelectric point of the λ1 light chain, *p*I ~4.5) in these variants explains the acidic shift in their IEF pattern. No other mutations were found when about 80% of the *V* regions of the variants were sequenced (data not shown).

Explanations of the *V–J* joining mechanism must be consistent with the above findings. The extra T at the *V–J* junction is probably derived from the region just 3′ of the λ1 *V* region (see Fig. 2), that is, the recombination point is moved by one nucleotide. To explain the deletions upstream of the actual join, we speculate that *V–J* recombinations have error-prone sealing and repair activities. We do not know how frequently small deletions accompany the joining process nor do we know whether the deletions can occur also at the 3′ side of the junction because we have analysed only a selected subpopulation of λ1 chains. Certainly, the one-base deletion does not always occur when an extra T is added at the *V–J* join. An aberrantly rearranged λ1 light-chain gene having an 'extra T' but lacking the one-base deletion has been described[5].

Clearly, more data are needed to establish whether the mechanism described here is an impotant source of diversity in antigen receptors which are formed by the joining of two separate gene segments.

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1. Tonegawa, S. *Nature* **302**, 575–581 (1983).
2. Freitas, A. A. & Coutinho, A. *J. exp. Med.* **154**, 994–999 (1981).
3. Takemori, T. & Rajewsky, K. *Eur. J. Immun.* **11**, 618–625 (1981).
4. Andersson, J., Coutinho, A. & Melchers, F. *J. exp. Med.* **147**, 1744–1754 (1978).
5. Hozumi, N. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 7019–7023 (1981).
6. Köhler, G. & Milstein, C. *Nature* **256**, 495–496 (1975).
7. Towbin, H., Staehlin,T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354 (1979).
8. Bothwell, A. L. M. *et al. Nature* **298**, 380–382 (1982).
9. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning, A Laboratory Manual* (1982).
10. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).

Induction of c-*fos* during myelomonocytic differentiation and macrophage proliferation

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Previous studies have suggested a role for c-*fos* in cellular differentiation in fetal membranes[1–3], haematopoietic cells[4,5] and teratocarcinoma stem cells[6]. In other cell types, such as fibroblasts, c-*fos* expression is normally very low, but is rapidly induced by peptide growth factors, implicating c-*fos* in growth control mechanisms[7–10]. Here, we show that the TPA (12-*O*-tetradecanoylphorbol-13-acetate)-induced macrophage-like differentiation of HL60 human promyelocytic precursor cells[11,12] is accompanied by the induction of both c-*fos* mRNA and protein within 15 min after treatment, suggesting a functional role for c-*fos* in this differentiation system. In quiescent terminally differentiated macrophages, expression of c-*fos* is inducible by the macrophage-specific growth factor colony-stimulating factor-1 (CSF-1)[13]. The kinetics of c-*fos* induction, however, are entirely different from those in growth factor-stimulated fibroblasts, supporting the view that the c-*fos* gene product may serve different functions in different cell types.

The human promyelocytic leukaemia cell line HL60 (ref. 11) can be induced by the tumour promoter TPA to differentiate into macrophage-like cells[12]. Previously, it has been shown that this differentiation is associated with a drastic reduction in the level of expression of c-*myc* mRNA[5,14] and an increase in the level of c-*fos* mRNA[5] at a time when differentiation is almost complete (that is, 48 h after induction). To investigate a possible involvement of c-*fos* in the induction of differentiation, the time course of c-*fos* expression following TPA treatment of HL60 cells was followed in detail. Figure 1*a*,*b* shows that c-*fos* mRNA was not detectable in normal HL60 cells, but treatment with 5×10^{-7} M TPA induced c-*fos* expression within 15 min. Maximum expression was observed 30–45 min after addition of TPA; thereafter, the level of c-*fos* mRNA decreased 3–5-fold and remained at this level for the following 48 h. In contrast, expression of c-*myc* mRNA did not change until 4 h after TPA addition, when expression fell by more than 20-fold (Fig. 1*a*).

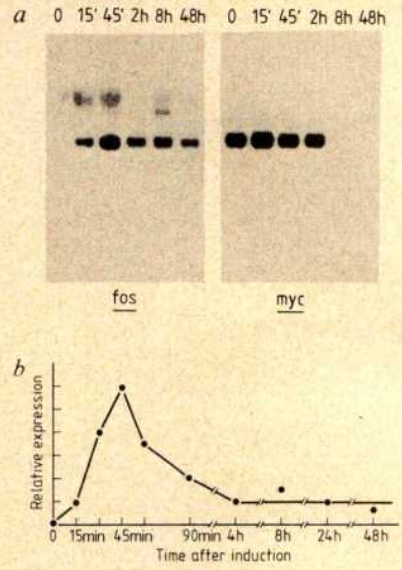


Fig. 1 Expression of c-*fos* and c-*myc* in TPA-induced differentiating HL60 cells. *a*, Northern blot analysis of total RNA (16 μg per lane) isolated at the indicated times after induction by 5×10^{-7} M TPA. Methods as described elsewhere[8]. Hybridization to v-*fos* and mouse c-*myc*-specific probes was performed as described[8]. *b*, Relative expression of c-*fos* plotted against time after TPA treatment. Quantitative evaluation of the Northern blot autoradiograms (similar to that shown in *a*) was done by comparing band intensities.

Differentiation of HL60 cells can also be induced by compounds other than TPA. A vitamin D_3 dihydroxy derivative, $1,25(OH)_2D_3$, induces monocyte differentiation[15], whereas treatment with retinoic acid gives rise to granulocytic cells[16]. In both cases, however, rapid induction of c-*fos* mRNA was not detectable (data not shown). In another study[4], it was reported that granulocyte–colony stimulating factor(G-CSF)-induced mouse myelomonocytic precursor cells express c-*fos* only at late stages of differentiation (that is, >2 days after induction). Taken together, these observations show that c-*fos* expression at an early stage is not an obligatory step in the differentiation of myelomonocytic or granulocytic cells. Rapid induction of c-*fos* expression seems to be specific for the TPA-induced pathway of monocytic differentiation.

Because c-*fos* protein has not yet been detected in human cells, we investigated whether the rapid induction of c-*fos* mRNA expression is associated with induction of c-*fos* protein, by immunoprecipitation of ^{35}S-labelled proteins from HL60 cells induced by TPA for various times. We used an antiserum directed against a synthetic peptide (designated 'M') common to v-*fos* and both mouse and human c-*fos* sequences[17]. It has been shown previously that this antiserum detects modified c-*fos* protein of relative molecular mass M_r 57,000–62,000 (57–62 K) which is complexed with a cellular protein, p39 (refs 8, 17–19;

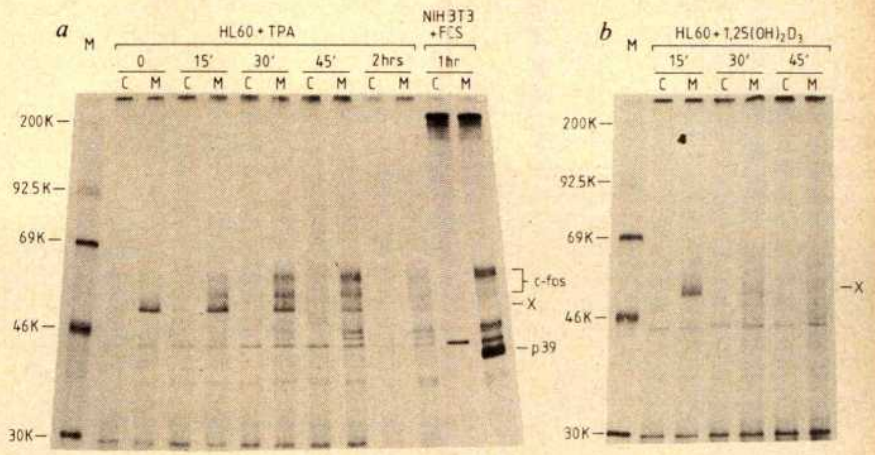


Fig. 2 Immunoprecipitation of c-*fos*-related protein from HL60 cells induced to differentiate by 50×10^{-8} M TPA (*a*) or 10^{-7} M $1,25(OH)_2D_3$ (*b*) for different times. C, Control serum (v-*fos* peptide-specific antiserum; V in ref. 17); M, *fos* peptide-specific antiserum[17]. For comparison, immunoprecipitation of c-*fos* protein from FCS-stimulated NIH 3T3 cells is also shown (*a*). With HL60 cells, immunoprecipitation was performed on lysates of ^{35}S-methionine-labelled cells denatured by boiling for 5 min in 0.5% SDS[17], thus no p39 is co-precipitated in these lanes. The X protein was not found in non-denatured lysates, and its level was subject to considerable experimental variation in denatured cell lysates, suggesting that it may be a cross-reacting protein that shares only very restricted antigenicity with the c-*fos* gene product.

Fig. 2*a*). Protein of very similar M_r was detected in the HL60 cells as early as 15 min after TPA induction. The levels of c-*fos* protein were maximal at 30–45 min, then decreased (Fig. 2*a*). The cellular p39 protein was not detected in this experiment because immunoprecipitation was performed on cell lysates denatured by boiling in SDS. Very similar results were obtained with a *fos*-specific tumour-bearing rat (TBR) serum[19,20] (data not shown). In agreement with RNA analyses, expression of c-*fos* protein was not detectable in $1,25(OH)_2D_3$-induced HL60 cells (Fig. 2*b*). Another group of proteins in the 45 K range increased concomitantly with c-*fos* in both TPA-induced HL60 cells and—as described previously[8]—in fetal calf serum (FCS)-stimulated NIH 3T3 cells. These proteins may be encoded by a c-*fos* related gene, such as r-*fos*, which, in growth factor-stimulated fibroblasts, is co-induced with c-*fos*[10]. Coincidentally, the *fos*-specific antiserum was raised against a peptide that is also contained in the r-*fos* protein[10,17]. A more precise definition of these proteins awaits peptide mapping analyses. Interestingly, a protein of $M_r \sim 55$ K (designated 'X' in Fig. 2) was detectable in denatured lysates of uninduced HL60 cells and disappeared rapidly after induction of differentiation by either TPA or $1,25(OH)_2$-D_3 (Fig. 2*a*,*b*). As c-*fos* RNA is not detectable in uninduced HL60 cells (Fig. 1*a*), the X protein apparently cannot be encoded by c-*fos*. Although this protein was also detected by the *fos*-specific TBR serum (data not shown) its nature remains obscure.

In fibroblasts, c-*fos* expression is rapidly inducible by growth factors and is therefore thought to be associated with growth regulation. Although macrophages are terminally differentiated cells, they still retain the ability to proliferate, unlike most other cell types. Therefore, it was of interest to investigate whether c-*fos* expression in mature macrophages might be correlated with growth. When primary cultures of bone marrow-derived macrophages were deprived of the macrophage-specific growth factor CSF-1 for 18 h (Fig. 3*a*) or kept at low serum concentrations for 24 h (0.5% FCS; data not shown), proliferation ceased and the cells became quiescent. This was accompanied by a marked reduction in levels of both c-*fos* and c-*myc* mRNA (Fig. 3*b*, and data not shown), suggesting that expression of these proto-oncogenes depends on the presence of growth factors (or on cellular proliferation). When CSF-1-deprived macrophages in the G_0 phase of the cell cycle were stimulated by addition of the growth factor, induction of c-*myc* was observed after 1 h, reaching a maximum at 2–4 h. Activation of c-*myc* was followed by an ~10-fold induction of c-*fos* mRNA levels, which remained high for the following 8 h (Fig. 3*b*). Regulation of c-*fos* expression in macrophages differs substantially from that in fibroblasts: (1) in contrast to fibroblasts[8], a high level of c-*fos* expression is evident in normally growing, asynchronous macrophages, and thus appears to be part of the normal cell cycle in these cells; (2) induction of c-*fos* by CSF-1 in macrophages occurs much later than in growth factor-stimulated

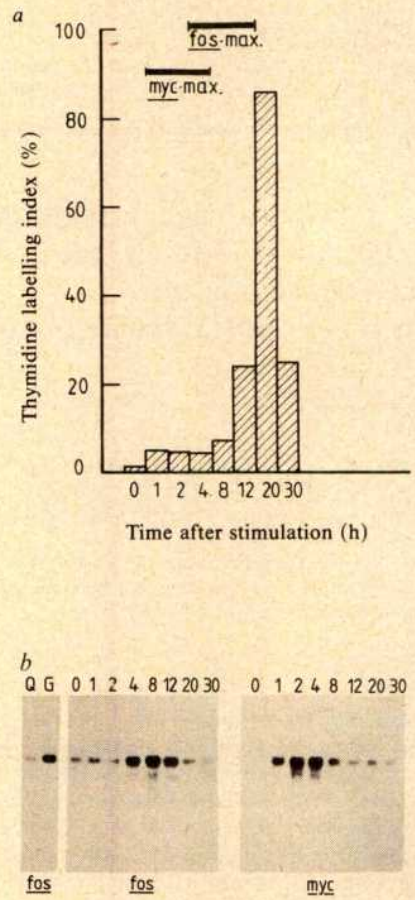


Fig. 3 *a*, Thymidine labelling index at the indicated times after CSF-1 induction of quiescent macrophages. Horizontal bars indicate the periods of maximal c-*fos* and c-*myc* expression, respectively, as observed in the experiment shown in *b*. *b*, Expression of c-*fos* and c-*myc* in quiescent versus growing or stimulated macrophages. Northern blot analysis of RNA from normally growing (G) versus CSF-1-deprived, quiescent (Q) mouse macrophages, and from quiescent macrophages stimulated with CSF-1 for the indicated times (0, 1, 2, 4, 8, 12, 20 and 30 h).

Methods. Primary cultures of bone marrow-derived macrophages from C3H/HeJ mice were established and grown as described previously[13]. Cultures were used at day 7 after establishment. At this time the population consisted of >98% macrophages. Quiescent macrophages were obtained by maintaining the culture for 18 h in CSF-1-free medium supplemented with 15% FCS. Stimulation was carried out by adding 3,000 units of partially purified CSF-1 (stage 1) per ml of medium. Purification of CSF-1 from mouse L-cell supernatant has been described previously[22]. One dish (175 cm^2) of macrophages was used for each time point.

fibroblasts, where it precedes activation of c-*myc*; (3) induction of c-*fos* in macrophages lasts considerably longer than in fibroblasts. While in stimulated fibroblasts c-*fos* induction appears to occur during the transition from the G_0 to G_1 phase of the cell cycle, expression in macrophages is observed during G_1 until mid-S phase. These findings further substantiate our hypothesis[6,8] that the c-*fos* gene product serves different functions in fibroblasts and macrophages.

TPA induces cell proliferation in fibroblasts[21] and differentiation in HL60 cells[12]. In both cases, rapid induction of c-*fos* mRNA and protein was observed (Figs 1, 2; ref. 7 and R. Bravo, J. Burckhardt, T.C. and R.M., unpublished observation). It is possible that the initial biochemical events induced by TPA are the same regardless of whether the ultimate consequence is differentiation or proliferation. Such initial events, in which *fos* may have a role, include the transduction of TPA-induced signals received at the cell membrane within the nucleus. In mature myelomonocytic cells, c-*fos* may be involved in biochemical pathways controlling proliferation and/or securing the survival of macrophages. In this context it is interesting that c-*fos* is induced by the growth factor CSF-1, which has been shown to be required for both growth and survival of macrophages[13].

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1. Müller, R., Slamon, D. J., Tremblay, J. M., Cline, M. J. & Verma, I. M. *Nature* **299**, 640–644 (1982).
2. Müller, R., Verma, I. M. & Adamson, E. D. *EMBO J.* **2**, 679–684 (1983).
3. Müller, R., Tremblay, J. M., Adamson, E. D. & Verma, I. M. *Nature* **304**, 454–456 (1983).
4. Gonda, T. J. & Metcalf, D. *Nature* **310**, 249–251 (1984).
5. Müller, R., Müller, D. & Guilbert, L. *EMBO J.* **3**, 1887–1890 (1984).
6. Müller, R. & Wagner, E. F. *Nature* **311**, 438–442 (1984).
7. Greenberg, M. E. & Ziff, E. B. *Nature* **311**, 433–438 (1984).
8. Müller, R., Bravo, R., Burckhardt, J. & Curran, T. *Nature* **312**, 716–720 (1984).
9. Kruijer, W., Cooper, J., Hunter, T. & Verma, I. M. *Nature* **312**, 711–716 (1984).
10. Cochran, B. H., Zullo, J., Verma, I. M. & Stiles, C. D. *Science* **226**, 1080–1082 (1984).
11. Collins, S. J., Gallo, R. C. & Gallagher, R. W. *Nature* **270**, 347–349 (1977).
12. Rovera, G., O'Brien, T. G. & Diamond, L. *Science* **204**, 868–870 (1979).
13. Tushinsky, R. J. *et al. Cell* **28**, 71–78 (1982).
14. Westin, E. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2490–2494 (1982).
15. McCarthy, D. M. *et al. Leukemia Res.* **7**, 51–55 (1983).
16. Reitsma, P. H. *et al. Nature* **306**, 492–494 (1983).
17. Curran, T., Van Beveren, C., Ling, N. & Verma, I. M. *Molec. cell Biol.* **5** (in the press).
18. Curran, T. & Teich, N. M. *Virology* **116**, 221–235 (1982).
19. Curran, T., Miller, A. D., Zokas, L. & Verma, I. M. *Cell* **36**, 259–268 (1984).
20. Curran, T. & Teich, N. M. *J. Virol.* **42**, 114–122 (1982).
21. Sivak, H. *J. cell. Physiol.* **80**, 167–174 (1972).
22. Stanley, E. R. & Guilbert, L. Y. *J. immun. Meth.* **42**, 253–284 (1981).

Increased neurovirulence associated with a single nucleotide change in a noncoding region of the Sabin type 3 poliovaccine genome

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Most of the small number of cases of poliomyelitis which occur in countries where Sabin's attenuated poliovirus vaccines are used are temporally associated with administration of vaccine and involve polioviruses of types 2 and 3 (ref. 1). Recent studies have provided convincing evidence that the Sabin type 2 and 3 viruses themselves may revert to a neurovirulent phenotype on passage in man[2–6]. We report here that a point mutation in the 5′ noncoding region of the genome of the poliovirus type 3 vaccine consistently reverts to wild type in strains isolated from cases of vaccine-associated poliomyelitis. Virus with this change is rapidly selected on passage through the human gastrointestinal tract. The change is associated with a demonstrable increase in the neurovirulence of the virus.

The complete nucleotide sequences of the genomes of the neurovirulent progenitor of the Sabin type 3 vaccine virus, P3/Leon/37, the Sabin vaccine virus itself, P3/Leon/$12a_1$b, and a virulent revertant strain from the brain of a fatal case of vaccine-associated poliomyelitis, P3/119 (ref. 7), have been determined[4,7–9]. The small number of sequence differences between these strains[4,7] suggested that the following mutations could be involved in attenuation and reversion: (1) a single nucleotide change at position 472 in the 5′ noncoding region of the genome; (2) nucleotide changes resulting in amino-acid changes in the capsid proteins; and (3) a single change at the end of the 3′ noncoding region. Only the nucleotide at position 472 correlated with the neurovirulent phenotype, being cytidine in P3/Leon/37 and P3/119, and uridine in the vaccine strain.

Table 1 Base at position 472, time of isolation, neurovirulence and temperature sensitivity of Sabin type 3 vaccine-derived strains of poliovirus

Virus	Base at position 472	Time of isolation after vaccination	Mean histological lesion score	rct marker test*
Sabin vaccine	U		0.36	>5.5(rct$^-$)
DM1	U	24 h	ND	ND
DM2	U	31 h	1.58	6.13 (rct$^-$)
DM3	U/C	35 h	ND	ND
DM4†	C	47 h	2.48	5.71 (rct$^-$)
DM38	C	18 days	ND	ND
DM119	C	3–4 weeks	3.34	0.25 (rct$^+$)

Mean histological lesion scores were determined using the standard WHO neurovirulence test[14]. The range of mean histological lesion scores of a type III attenuated reference strain in eight tests carried out over the past 2 yr is 0.43–0.79. ND, not determined.

* The rct marker (replicative capacity at temperature −40 °C) test shows the logarithmic difference between virus titres grown at 35 °C and at 40 °C[15]. Viruses showing reductions of log 5.0 or more in titre are considered to be temperature-sensitive (rct$^-$).

† The sequences of the structural coding region and the 5′ noncoding region of DM4 were determined as described previously[10]. The extreme 3′ end of the RNA was sequenced by synthesizing complete complementary DNA copies of the viral RNA, annealing a synthetic oligonucleotide primer to its 5′ end and sequencing by the dideoxy method using the Klenow fragment of *Escherichia coli* DNA polymerase I[13].

We therefore examined the frequency with which the mutation to cytidine at base 472 occurred in viruses from vaccine-associated cases of poliomyelitis, and assessed its effect on the phenotype of the virus.

Twelve strains of poliovirus type 3 from a collaborative study of vaccine-associated poliomyelitis performed by the World Health Organization (WHO)[9] were examined in addition to P3/119. All the strains had been shown to be genetically related to the Sabin vaccine virus by T1 oligonucleotide mapping[2,3,6], and six had been found to be highly neurovirulent[9]. In all 12 strains, the nucleotide at position 472 of the genome was cytidine, as shown by primer extension sequencing[10] using a synthetic primer which annealed to the viral RNA from position 510 to 522 (data not shown). In contrast, the nucleotide at this position in two batches of vaccine was uridine, confirming our previous findings[4,8,9] and those of others[11]. The consistency with which cytidine was detected at position 472 amongst the vaccine-derived revertant viruses suggested that this nucleotide might have an effect on the virulence of the virus.

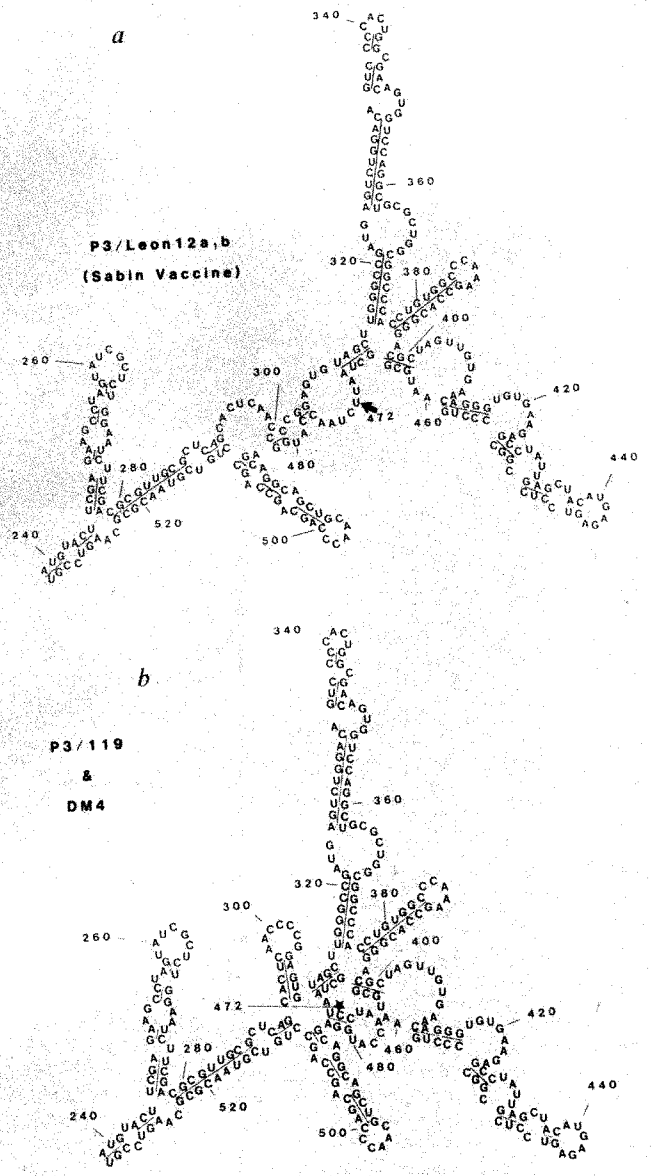


Fig. 1 Predicted secondary structure of RNA (bases 240–540, approximately) from type 3 Sabin poliovirus vaccine strain (*a*) and type 3 revertant strain P3/119 (*b*) based on the improved RNZ folding algorithm of Jacobson *et al.*[18]. We used a recent display algorithm which allows easy visual comparison of similar sequences[19].

It is known that Sabin type 3 vaccine virus increases in neurovirulence on passage in the human gut[12]. Therefore, we attempted to determine whether this loss of attenuation is associated with the substitution at position 472. After oral administration of the trivalent Sabin vaccine to a 4-month-old infant, we collected every faecal sample and attempted to isolate the virus from each. The genomes of several of the successive type 3 isolates were sequenced through position 472 (Table 1). DM1 and DM2, isolates from the first two faecal samples obtained 24 and 31 h after vaccination respectively, possessed U at position 472, as in the vaccine virus. The third isolate, DM3, from a faecal sample collected at 35 h, contained a mixture of viruses with either U or C at position 472. The fourth isolate (DM4), from a sample collected just 47 h after vaccination, and all subsequent isolates sequenced appeared to be homogeneous for C at position 472. These findings suggest strongly that replication of the vaccine virus in the human gut rapidly selects for viruses containing C at position 472 of the genome. No other changes, including those with significance for reversion[4,13], were detected when the entire structural coding region and the 3′ and 5′ noncoding regions of DM4 were sequenced (Table 1).

To assess the significance of the mutation at position 472 for neurovirulence, DM2 and DM4 were assayed in a standard WHO neurovirulence test[14]. DM4 was found to be significantly more neurovirulent than the vaccine strain with respect to both the histological lesions in the central nervous system (CNS) (Table 1) and the paralysis induced (data not shown), although it was not as neurovirulent as the revertant P3/119 (Table 1). The second isolate, DM2, had a higher lesion score than the Sabin vaccine strain. Our interpretation of this finding is that DM2 probably contained a small proportion of virus with C at position 472 which was undetectable by the sequencing methods used.

DM4 was found to be temperature-sensitive (rct$^-$) (Table 1), as is the Sabin vaccine virus[15]. In contrast, the revertant P3/119 was rct$^+$, as were all other revertants tested[9]. Therefore, it seems that reversion at position 472 alone does not account for the rct$^+$ phenotype of revertant strains.

As the extremely rapid selection of virus having cytidine at position 472 could be atypical, daily faecal samples were collected from three other vaccinated infants; 4 days after vaccination all three subjects were excreting virus having C at position 472 (Table 2).

Table 2 Base at position 472 of the poliovirus genome in primary vaccinees

Day post-vaccination	Vaccinee KT1	KT2	KT3
1	U	*	U
2	U/C	*	U
3	C	*	C
4	C	C	C

Faecal samples were taken daily from three vaccinated infants less than 1 yr old, who received vaccine of the same origin as DM (Table 1).
* Isolates of poliovirus type 3 not available.

Virus with C at position 472 is thus selected for rapidly and consistently following administration of vaccine, the short period of time before the appearance of the first isolate with C probably representing only a few cycles of virus replication.

The basis of the selective advantage conferred by the presence of C at position 472 and the function of the 5′ noncoding region are unclear. The degree of sequence homology between the three poliovirus serotypes in the 5′ noncoding region is high (74%)[16]. Moreover, this is the region that is most conserved between the genomes of polioviruses and rhinovirus type 14 (ref. 17). This strong conservation suggests that the region has functional significance. One possibility is that some of the short open reading frames present in this region are functional and that their products remain undetected. Other possible functions of this 5′ region include replicase binding on the negative-strand RNA, packaging of RNA into new virus capsids, uncoating and control of the initiation of translation. Such functions could conceivably depend on RNA secondary structure.

Figure 1 shows a prediction of the secondary structure of the RNA[18,19] of the Sabin vaccine virus and of isolate P3/119; the change from U to C at position 472 in P3/119 causes a substantial rearrangement, so that two additional stem-and-loop structures are formed. A similar structure to that shown for P3/119 was also predicted for the virulent vaccine progenitor P3/Leon/37.

We have shown that for poliovirus type 3, a single nucleotide change of U to C at position 472 in the noncoding region of the viral genome provides a selective advantage for growth in the human gut. This change is associated with an increase in neurovirulence of the virus although it does not, on its own, give rise to a fully neurovirulent phenotype. Neither the nature of the selective environment nor the basis of the selective advantage conferred by the change is known.

It is of interest that, according to the particular RNA folding algorithm used in the present study, the U to C mutation appears to have a profound effect on the predicted secondary structure

of the virus RNA, but it remains to be established whether the secondary structures presented and the differences between them have any biological significance. It is possible that analogous changes are important in the attenuation of poliovirus types 1. The type 1 vaccine strain, P1/LSc, shows seven differences compared with its neurovirulent parent, P1/Mahoney, in the noncoding region of its genome[16], and consequently some major rearrangements in the secondary structure of the RNA were predicted by this particular algorithm, including some in the region of position 472.

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1. Assaad, F. & Cockburn, W. C. *Bull Wld Hlth Org.* **60**, 231-242 (1982).
2. Minor, P. D. *J. Virol.* **34**, 73-84 (1980).
3. Minor, P. D. *J. gen. Virol.* **59**, 307-317 (1982).
4. Cann, A. J. *et al. Nucleic Acids Res.* **12**, 7787-7792 (1984).
5. Cossart, Y. E. *Br. med. J.* **1**, 162-163 (1977).
6. Kew, O. M., Nottay, B. K., Hatch, M. H., Nakano, J. H. & Obijeski, J. F. *J. gen. Virol.* **56**, 337-347 (1981).
7. Stanway, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 1539-1543 (1984).
8. Stanway, G. *et al. Eur. J. Biochem.* **135**, 529-533 (1983).
9. WHO Collaborative Study. *J. biol. Standard.* **9**, 163-184 (1981).
10. Evans, D. M. A., Minor, P. D., Schild, G. C. & Almond, J. W. *Nature* **304**, 459-462 (1983).
11. Toyoda, H. *et al. J. molec. Biol.* **174**, 561-585 (1984).
12. Magrath, D. I., Boulger, L. R. & Hartley, E. G. *10th Eur. Symp. Poliomyelitis*, 334-345 (EAP, 1964).
13. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
14. *Tech. Rep. Ser. Wld Hlth Org.* **687**, 130 (1983).
15. *Tech. Rep. Ser. Wld Hlth Org.* **687**, 134 (1983).
16. Nomoto, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 5793-5797 (1982).
17. Stanway, G., Hughes, P. J., Mountford, R. C., Minor, P. D. & Almond, J. W. *Nucleic Acids Res.* **12**, 7859-7875 (1984).
18. Jacobson, A. B., Good, L., Simonetti, J. & Zuker, M. *Nucleic Acids Res.* **12**, 45-52 (1984).
19. Shapiro, B. A., Maizel, J. V., Lipkin, L. E., Currey, K. & Whitney, C. *Nucleic Acids Res.* **12**, 75-88 (1984).

Suppression of leukaemia virus pathogenicity by polyoma virus enhancers

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The long terminal repeats (LTRs) of retroviruses contain sequences necessary for the initiation and termination of retroviral transcription. These sequences include promoter elements, transcriptional termination signals and transcriptional enhancer elements. The enhancer elements of Moloney murine leukaemia virus (M-MuLV) are localized in a tandemly repeated region (~75 base pairs (bp) long), which lies 5′ to the CAT and TATA promoter elements in the U3 region of the LTR (see Fig. 1)[1]. We have shown that the tandem repeats are required both for LTR promoter activity, as measured by transient expression assays, and for biological activity, as measured by production of infectious virus[2]. Furthermore, they can be replaced by transcriptional enhancers from the F101 host-range mutant of polyoma virus without loss of function. We report here that the addition of the polyoma (PyF101) enhancers to the M-MuLV LTRs (either with or without the M-MuLV tandem repeats) results in complete loss of viral leukaemogenicity, even though the virus can replicate to high titres in tissue culture fibroblasts and can establish infection in animals.

Figure 1*A* shows the modified M-MuLV LTRs used in the present study. Mo+PyF101 was constructed by inserting the PyF101 enhancer element into the wild-type M-MuLV LTR at

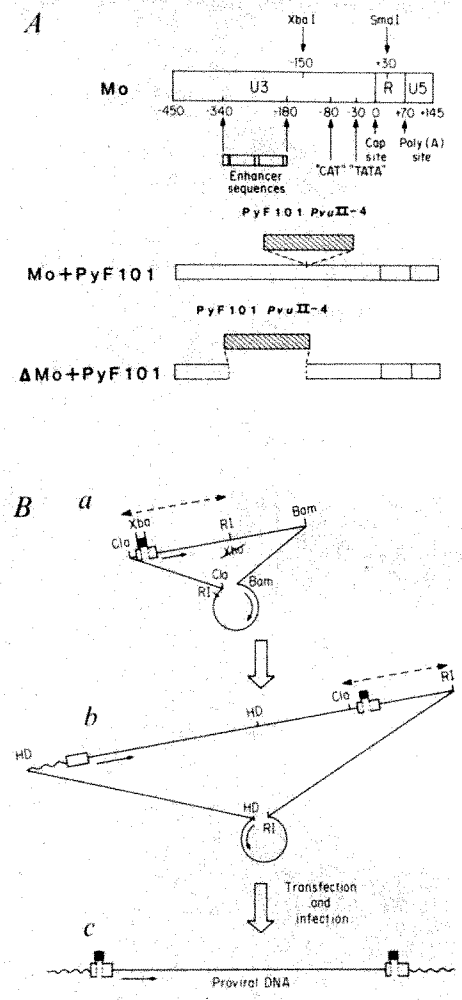


Fig. 1 *A*, M-MuLV LTRs used for tests of infectivity and pathogenicity. Mo, The wild-type M-MuLV LTR from a clone of circularly permuted proviral DNA, pMLV-1a[1,24]; the sequences important in proviral transcription are indicated. The tandem repeats, implicated as enhancer elements[14,25], are located upstream (−341 to −182) of the promoter region. Mo+PyF101, LTR containing the *Pvu*II-4 fragment of mutant polyoma virus PyF101 (ref. 26) inserted into the M-MuLV LTR at the *Xba*I site at position −150, 30 bp 3′ of the M-MuLV tandem repeats. Mo+PyF101 was constructed by first adding *Xba*I linkers to the 199-bp *Pvu*II-4 fragment[2], then inserting it into M-MuLV at the *Xba*I site. ΔMo+PyF101, LTR containing the PyF101 *Pvu*II-4 fragment inserted into an M-MuLV LTR in which the tandem repeats (−335 to −150) have been deleted (see ref. 2 for details of construction). The orientation of the PyF101 *Pvu*II-4 fragment relative to the Moloney promoter is identical in ΔMo+PyF101 and Mo+PyF101. ***B***, The strategy used to construct M-MuLVs with modifications in the U3 region of the LTR. The *Cla*I to *Eco*RI fragment of ΔpMLV-C/R/B+PyF101 (*a*) was used to construct a complete M-MuLV recombinant DNA provirus containing a 3′ ΔMo+PyF101 LTR (*b*) (see ref. 2 for details). The construct containing the Mo+PyF101 LTR was produced in a similar fashion. As the LTR alterations were all located within the U3 region, transfection of these constructs and subsequent infection of neighbouring cells would result in proviruses containing modified LTRs at both their 5′ and 3′ ends (*c*).

−150 bp, between the M-MuLV tandem repeats and the M-MuLV promoter. ΔMo+PyF101 comprises an insertion of the PyF101 enhancer into an M-MuLV LTR from which the M-MuLV enhancer elements (from −335 to −150 bp with respect to the RNA cap site) have been deleted[2]. The biological activity of the LTRs was assessed by constructing a plasmid containing the complete M-MuLV proviral DNA sequence, with the altered LTR replacing the 3′ LTR, transfecting this plasmid into NIH 3T3 cells and assaying for infectious virus[2] (see Fig. 1*B* for a

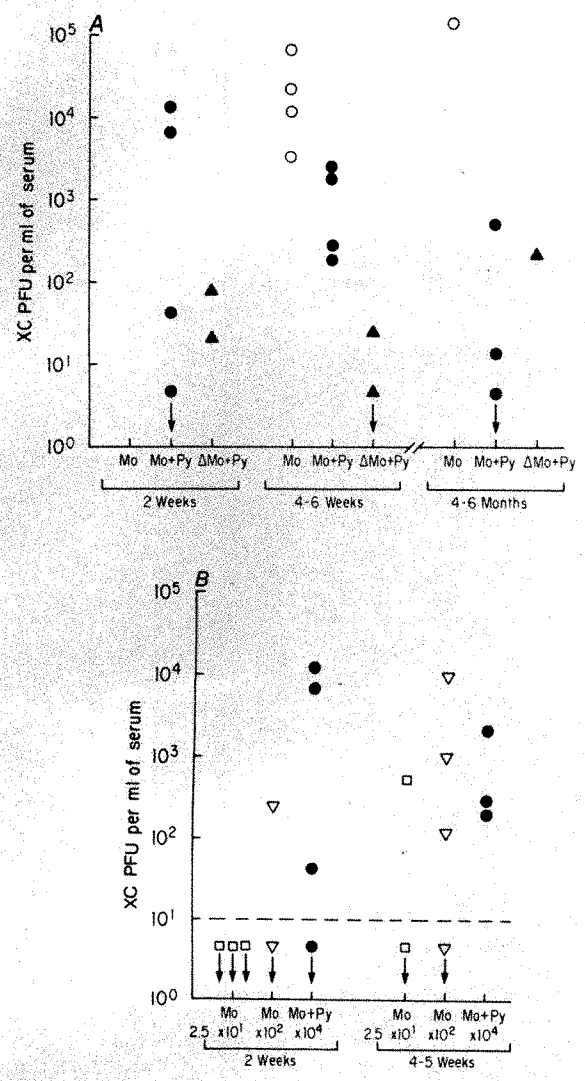


Fig. 2 Levels of circulating infectious virus in inoculated animals. Serum samples from mice injected i.p. 1–5 days after birth with either wild-type or polyoma-containing M-MuLVs were assayed by infection and titration on NIH 3T3 cells using the UV-XC assay[22]. Each data point in *A* and *B* shows the titration results obtained for a different sacrificed animal. Data points with downward-pointing arrows represent animals having virus levels undetectable in this assay ($<10^1$ XC PFU per ml of serum). *A*, Levels of virus in animals inoculated with 2.5×10^4 XC PFU of wild-type (○), Mo+PyF101 (●) or ΔMo+PyF101 (▲) M-MuLVs. Titration results are given for animals at 2 weeks, 4–6 weeks, and 4–6 months after injection. *B*, Levels of virus in animals inoculated with 2.5×10^1 XC PFU of wild-type M-MuLV (□), 2.5×10^2 XC PFU of wild-type M-MuLV (▽), or 2.5×10^4 XC PFU of Mo+PyF101 M-MuLV (●). Results are shown for animals 2 weeks and 4–5 weeks post-injection.

brief outline of these procedures). Infectious virus containing either of the altered LTRs could be recovered (designated Mo+PyF101 M-MuLV and ΔMo+PyF101 M-MuLV). Note that the alterations in these viruses lie in regions that do not encode protein, thus the virus particles are identical to wild-type M-MuLV.

To assess the relative infectivities of the ΔMo+PyF101 and Mo+PyF101 viruses, virus produced by the transfected cultures was infected onto NIH 3T3 cells, which were then transferred until confluently infected. The amount of infectious virus produced by the cultures was assayed by titration of tissue culture supernatants on NIH 3T3 cells (Table 1). Cells infected with the two PyF101-containing viruses produced high amounts of infectious virus, although less than the amount produced by wild-type M-MuLV. As quantified by the reverse transcriptase

Table 1 Biological activity of M-MuLV containing altered LTRs

LTR	Titre of infectious virus	No. of virus particles (reverse transcriptase assay)	Infectivity-to-particle ratio
Mo	1.1×10^7	4.4×10^6	2.5
Mo+PyF101	1.6×10^6	3.0×10^6	0.53
ΔMo+PyF101	5.0×10^5	3.1×10^6	0.16

Virus released from NIH 3T3 cells transfected with the M-MuLV proviral constructs containing altered LTRs was infected onto fresh NIH 3T3 cells. The freshly infected cells were transferred four times, after which all cells were infected; 24-h tissue culture supernatants were collected from 10^6 cells and assayed for concentration of infectious virus and for the number of virus particles present. The titre of infectious virus was quantified by titration of supernatants onto NIH 3T3 cells using the rat XC cell syncitial assay[22]; the values shown represent XC PFU ml^{-1}. The number of virus particles present was determined by the reverse transcriptase assay, using poly(rA)/oligo(dT) as exogenously added template/primer[25]; the values shown represent c.p.m. dTTP incorporated in a 75-min reaction for virus collected from 1 ml of tissue culture supernatant. Typical values for dTTP incorporation ranged from 4.0×10^5 to 6.5×10^5 c.p.m.; supernatant from uninfected NIH 3T3 cells yielded approximately 1% as much dTTP incorporation. Infectivity-to-particle ratios were calculated by dividing the infectious virus titre by the reverse transcriptase activity for each of the infected cell tissue culture supernatants.

assay (Table 1), cells infected with ΔMo+PyF101 and Mo+PyF101 M-MuLVs produced almost the same number of virus particles as wild-type M-MuLV. To compare directly the infectivity of the polyoma-containing MuLVs with that of wild-type M-MuLV, infectivity-to-particle ratios were calculated by dividing the infectious virus titre by the reverse transcriptase activity present in each of the infected tissue culture supernatants (Table 1). Compared with wild-type M-MuLV, Mo+PyF101 virus showed a slight (fivefold) decrease in specific infectivity and ΔMo+PyF101 virus a somewhat greater (15–30-fold) decrease. Thus, the polyoma-containing M-MuLVs are able to productively infect NIH 3T3 fibroblasts, and show only slightly decreased infectivity compared with wild-type M-MuLV.

The pathogenicity of wild-type, ΔMo+PyF101 and Mo+PyF101 M-MuLVs was assessed by inoculating neonatal (1–3-day-old) NIH Swiss mice intraperitoneally (i.p.) with equal infectious units ($\sim2.5\times10^4$ XC plaque-forming units, PFU) of each virus, then monitoring the mice for appearance of disease; any moribund animals were autopsied. Injection of six neonates with wild-type M-MuLV resulted in development of typical M-MuLV thymic leukaemia and death in all of the animals. Diseased animals showed enlarged thymuses, spleens or lymph nodes. The mean time until the disease became apparent was 3 months, with all mice developing the disease before 4 months. These results are in agreement with those obtained by us and others for M-MuLV[3]. In contrast, there was no evidence of disease in 10 neonates (from two litters) injected with Mo+PyF101 M-MuLV and in 17 neonates (from two litters) injected with ΔMo+PyF101 M-MuLV, even 12–13 months after injection. Also, autopsies of animals inoculated with either virus showed no signs of abnormality or malignancy. These results suggest that Mo+PyF101 and ΔMo+PyF101 M-MuLVs are non-leukaemogenic, even when inoculated at high titre.

One possible explanation for the lack of pathogenicity of the altered viruses is that they are unable to establish infection *in vivo*, even though they can replicate in tissue culture. To examine this possibility, circulating levels of infectious virus in inoculated animals were measured by titration of the blood serum on NIH 3T3 cells (Fig. 2*A*). Infectious XC-positive MuLV was recovered from the great majority of mice (13 out of 16) inoculated with Mo+PyF101 M-MuLV or ΔMo+PyF101 M-MuLV, at time points between 2 weeks and 6 months after injection. XC-positive virus (although at low titre) was recovered even 5–6

months after injection with the polyoma-containing M-MuLVs, which further emphasizes that these viruses were able to establish and sustain multiple rounds of infection in the inoculated neonates. The low titres of virus present at later time points in Mo+PyF101 M-MuLV-injected animals (compared with animals inoculated with wild-type M-MuLV) may have resulted from an immune response in the absence of any malignant growth of infected thymoma cells.

Even though the polyoma-containing viruses were clearly able to establish infection *in vivo*, it was conceivable that the lack of leukaemogenicity might have resulted from a quantitative defect in the level of infection established. Multiple rounds of infection in the animal are probably necessary for leukaemogenesis by M-MuLV, shown by the eventual appearance of 'mink cell focus-inducing' recombinant MuLVs[3,4], and by the site specificity of proviral integration in tumour cells[5-8]. To investigate this possible defect in more detail, neonatal mice were inoculated with lower levels of wild-type M-MuLV which were still sufficient to induce leukaemia (2.5×10^1 and 2.5×10^2 XC PFU per animal) and the circulating levels of M-MuLV were compared with those in animals inoculated with concentrated Mo+PyF101 M-MuLV (Fig. 2*B*). At 2 weeks post-injection, the levels of viraemia in animals inoculated with the diluted stocks of wild-type M-MuLV were significantly lower (undetectable in several animals) than in animals inoculated with concentrated Mo+PyF101 M-MuLV. Even at 4–5 weeks after injection, the increased levels of viraemia in animals inoculated with wild-type M-MuLV were only equivalent to those in animals inoculated with Mo+PyF101 M-MuLV. Thus, the level of infection in the Mo+PyF101 M-MuLV-inoculated animals would have been more than sufficient to cause disease if the virus had been wild-type. Animals inoculated with ΔMo+PyF101 M-MuLV also showed equivalent or higher levels of viraemia at 2 weeks post-inoculation than the animals inoculated with the diluted M-MuLV stocks.

The virus recovered from a 5-month animal inoculated with Mo+PyF101 M-MuLV was further characterized, since it was possible that recombination within the animal might have given rise to virus different from the input Mo+PyF101 M-MuLV. RNA and high relative molecular mass DNA were extracted from NIH 3T3 cells uniformly infected with the recovered virus and analysed by RNA dot-blot and DNA Southern blot analysis, respectively (Fig. 3*A*, *B*). RNA from the infected cells hybridized with a nick-translated PyF101 probe, confirming that M-MuLV containing the Mo+PyF101 LTR was still present in the animal 5 months after injection (Fig. 3*A*). DNA from cells infected with the recovered virus was digested with *Pvu*II and Southern blot hybridization was performed using an M-MuLV probe which detects a 1.88-kilobase (kb) fragment extending from −0.45 kb (relative to the cap site) in the Mo+PyF101 LTR to a *Pvu*II site at 1.43 kb on the proviral map (Fig. 3*C*). This *Pvu*II fragment is 199 bp larger in the Mo+PyF101 provirus than the equivalent fragment from wild-type M-MuLV provirus (1.68 kb) as a result of the insertion of the PyF101 sequences. Figure 3*B* shows that the *Pvu*II fragment in cells infected with the recovered virus was the same size as that in cells infected with the original Mo+PyF101 M-MuLV. Furthermore, no *Pvu*II fragments of different size were observed, indicating that no new M-MuLVs with recombinant LTRs arose in the animals inoculated with Mo+PyF101 M-MuLV.

These results suggest that the presence of polyoma virus enhancer sequences in the M-MuLV LTR abolishes the leukaemogenicity of the virus, even though it is still able to replicate in tissue culture fibroblasts and in the animal. The loss of pathogenicity did not result from an inability to establish infection in the animal, suggesting rather that it may reflect a change in tissue tropism of the virus resulting from addition of the polyoma enhancer sequences. The loss of leukaemogenicity may be a result of the polyoma-containing viruses being less able to infect the target cell(s) involved in development of M-MuLV lymphoma. There is accumulating evidence that certain transcriptional regulatory elements, including enhancers,

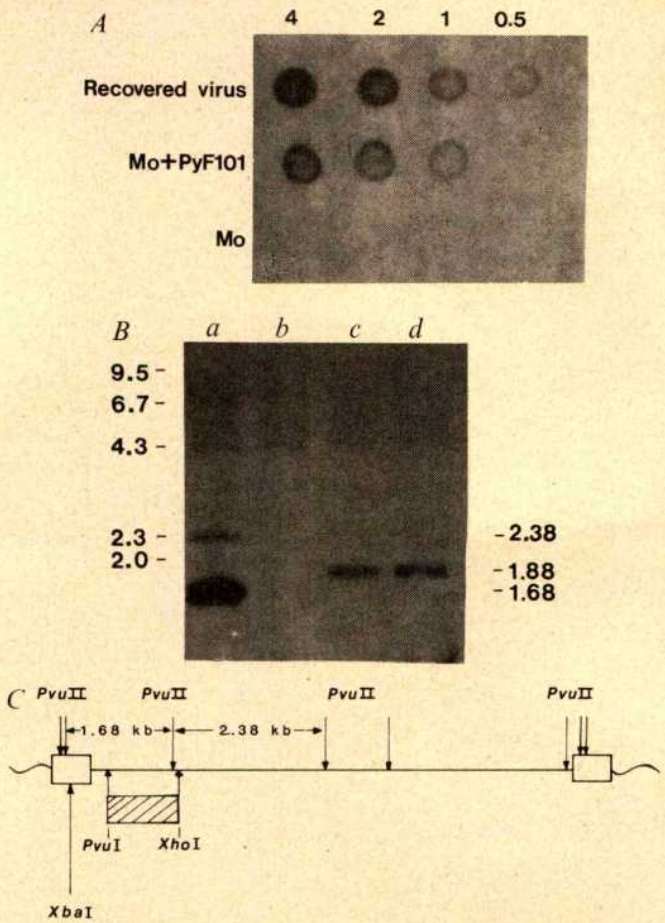


Fig. 3 Characterization of virus recovered from an animal inoculated with Mo+PyF101 M-MuLV. ***A***, RNA dot-blot hybridization[27] of total cytoplasmic RNA[28] isolated from NIH 3T3 cells confluently infected with wild-type M-MuLV (Mo), Mo+PyF101 M-MuLV, or virus recovered from the bloodstream of a mouse 5 months after inoculation with Mo+PyF101 M-MuLV (recovered virus). The amounts of RNA spotted onto the nitrocellulose sheet[27] were as shown (4, 2, 1 and 0.5 μg). The sheet was hybridized with ^{32}P-labelled nick-translated PyF101 *Pvu*II-4 fragment, and subjected to autoradiography. The probe hybridized with RNA from cells infected with Mo+PyF101 and with recovered virus, but not with RNA from cells infected with wild-type M-MuLV. RNA from all three cell lines hybridized with a labelled nick-translated M-MuLV probe (data not shown). ***B***, Southern blot hybridization of *Pvu*II-digested genomic DNA of high relative molecular mass, isolated from NIH 3T3 cell lines infected with wild-type M-MuLV (*a*), Mo+PyF101 M-MuLV (*d*) or recovered virus (*c*), and from uninfected NIH 3T3 cells (*b*). The Moloney cell line used in this experiment, M-MuLV clone A9, has been described previously[23]. Isolation of genomic DNA and blot-transfer hybridization were performed as described elsewhere[29,30]. The hybridization probe was a 1.1-kb *Pvu*I to *Xho*I fragment from 0.42 to 1.55 kb, relative to the cap site, on the proviral map (*C*). Hybridization with *Pvu*II-digested M-MuLV clone A9 DNA revealed two fragments of 1.68 and 2.38 kb (*a*), which were not present in *Pvu*II-digested uninfected NIH 3T3 DNA (*b*). The weakly hybridizing fragments (~4–9 kb) present in both uninfected and infected NIH 3T3 cells represented endogenous M-MuLV proviral sequences present in uninfected mouse cells. Insertion of the 199-bp PyF101 *Pvu*II-4 fragment in the Mo+PyF101 LTR increased the size of the 1.68-kb M-MuLV fragment to 1.88 kb, as predicted (*d*). The 2.38-kb M-MuLV fragment, although difficult to identify in *d*, was recognizable in the original autoradiogram. The weak hybridization to the 2.38-kb fragment was expected, as only a small fraction of the nick-translated M-MuLV *Pvu*I to *Xho*I restriction fragment could hybridize with the 2.38-kb *Pvu*II fragment (see *C*). The hybridization pattern obtained for cells infected with the recovered virus (*c*) was indistinguishable from that of Mo+PyF101 M-MuLV-infected cells (*d*). ***C***, The *Pvu*II restriction map of an integrated wild-type M-MuLV provirus, and the relative position of the nick-translated *Pvu*I to *Xho*I probe (hatched bar). The *Xba*I site in the LTR is also indicated.

can confer tissue specificity of expression[2,9-14]; in fact, recent reports suggest that enhancer sequences in the LTR determine the thymotropism and pathogenicity of murine leukaemia viruses[15-21].

We have found that loss of leukaemogenicity occurs not only for an M-MuLV LTR in which the M-MuLV tandem repeats have been replaced by the PyF101 enhancer (ΔMo+PyF101), but also for an M-MuLV LTR in which PyF101 enhancers have been added to a wild-type LTR still containing the M-MuLV enhancers (Mo+PyF101). The behaviour of Mo+PyF101 M-MuLV suggests that the PyF101 enhancer sequences exerted a dominant effect over the M-MuLV enhancer sequences present in the Mo+PyF101 LTR.

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1. Van Beveren, C., Rands, E., Chattopadhyay, S. K., Lowy, D. R. & Verma, I. M. *J. Virol.* **41**, 542-556 (1982).
2. Linney, E., Davis, B., Overhauser, J., Chao, E. & Fan, H. *Nature* **308**, 470-472 (1984).
3. Vogt, M. *Virology* **93**, 226-236 (1979).
4. Hartley, J. W., Wolford, N. K., Old, L. J. & Rowe, W. P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 789-792 (1977).
5. Li, Y., Holland, C. A., Hartley, J. W. & Hopkins, N. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6808-6811 (1984).
6. Corcoran, L. M., Adams, J. M., Dunn, A. R. & Cory, S. *Cell* **37**, 113-122 (1984).
7. Steffen, D. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2097-2101 (1984).
8. Cuypers, H. T. *et al. Cell* **37**, 141-150 (1984).
9. Banerjii, J., Olson, L. & Schaffner, W. *Cell* **33**, 729-740 (1983).
10. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717-728 (1983).
11. Mercola, M., Wang, X.-F., Olsen, J. & Calamme, K. *Science* **221**, 663-665 (1983).
12. Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. *Nature* **306**, 557-561 (1983).
13. Chen, I. S. Y., McLaughlin, J. & Golde, D. W. *Nature* **309**, 276-279 (1984).
14. Laimins, L. A., Khoury, G., Gorman, C., Howard, B. & Gruss, P. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6453-6457 (1982).
15. DesGroseillers, L., Rassart, E. & Jolicoeur, P. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4203-4207 (1983).
16. Chatis, P. A. *et al. J. Virol.* **52**, 248-254 (1984).
17. Lenz, J. *et al. Nature* **308**, 467-470 (1984).
18. Celander, D. & Haseltine, W. A. *Nature* **312**, 159-162 (1984).
19. DesGroseillers, L. & Jolicoeur, P. *J. Virol.* **52**, 448-456 (1984).
20. DesGroseillers, L. & Jolicoeur, P. *J. Virol.* **52**, 945-952 (1984).
21. Oliff, A., Signorelli, K. & Collins, L. *J. Virol.* **51**, 788-794 (1984).
22. Rowe, W. P., Pugh, W. E. & Hartley, J. W. *Virology* **42**, 1136-1139 (1970).
23. Fan, H., Jaenisch, R. & MacIsaac, P. *J. Virol.* **28**, 802-809 (1978).
24. Berns, A. J. M. *et al. J. Virol.* **36**, 254-263 (1980).
25. Jolly, D. J., Esty, A. C., Subramani, S., Friedmann, T. & Verma, I. M. *Nucleic Acids Res.* **11**, 1855-1872 (1983).
26. Fujimura, F. K., Deininger, P. L., Friedmann, T. & Linney, E. *Cell* **23**, 809-814 (1981).
27. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
28. Fan, H. & Baltimore, D. *J. molec. Biol.* **80**, 93-117 (1973).
29. Bacheler, L. T. & Fan, H. *J. Virol.* **30**, 657-667 (1979).
30. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).

Expression of enhanced levels of small RNA polymerase III transcripts encoded by the *B2* repeats in simian virus 40-transformed mouse cells

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Although specific viral genes are known which are sufficient to transform certain types of cells, viral transformation has been shown to be accompanied by a change in the level of expression of cellular genes. Assuming that this latter class of genes is involved in some way in the establishment of the transformed state, we and others[1,2] have sought to characterize them. To this end, we constructed a complementary DNA library from a simian virus 40 (SV40)-transformed mouse cell line and screened this library for induced genes. Here we focus on the characterization of a specific cDNA clone called 10λ5. We show that the clone 10λ5 contains a *B2* repeat[3,4], and that the levels of the small heterogeneously sized *B2* cytoplasmic RNAs homologous to 10λ5 are enhanced in transformed cells. Our studies show that these RNAs are a specific class of polymerase III transcripts containing a highly conserved 5′ end. SV40 transformation results, therefore, in the activation of specific polymerase III transcripts as well as specific polymerase II transcripts.

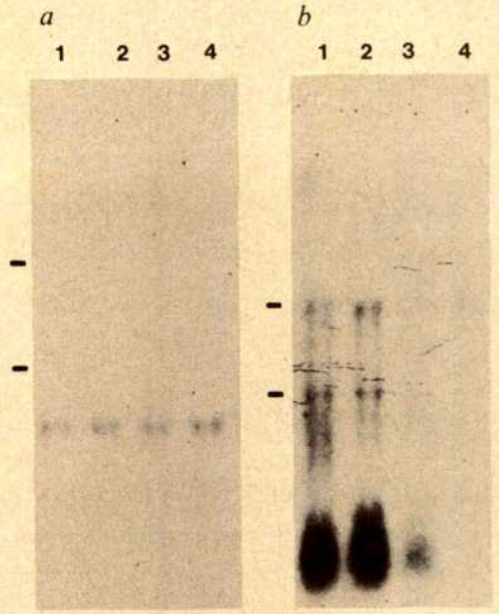


Fig. 1 Northern blot analysis of transcripts with homology to 8λ2, a non-differentially expressed cDNA clone that serves as a control (*a*), and 10λ5 (*b*). The lanes contain RNA from cells grown to different densities. Each lane contains 20 μg of cytoplasmic RNA. The lanes are: 1, SVLTR-1[22], cell density per 90 cm plate = 2.3×10^6; 2, SVLTR-1, cell density 18×10^6; 3, NIH 3T3, cell density, 2.7×10^6; 4, NIH 3T3, cell density, 5.5×10^6. Lanes 1 and 3 represent non-confluent cells and lanes 2 and 4 represent confluent cells. The positions of the 28S and 18S rRNAs are represented by lines on the left of each panel. In overexposed autoradiograms of total cytoplasmic RNA, some hybridization to both 18S and 28S RNA is detectable. The levels of the small RNAs homologous to 10λ5 shown in *b* are about fivefold greater in the transformed cell than in the non-confluent parental cell and about 20-fold greater in the transformed cell than in the confluent parental cell.

Methods. To isolate cDNA clones representing differentially expressed genes such as 10λ5, a plus/minus screen was performed on ≈10,000 phage from the SVLTR-1 λgt10 cDNA library. The cDNA probes used in the screening represented the poly(A)$^+$ RNA of either the NIH 3T3 or SVLTR-1 cells. Those cDNAs that consistently revealed a differential expression were isolated, subcloned into pBR322 and used as probes of Northern blots. Cells were grown in Dulbecco's modified minimal essential medium (DMEM) containing 10% calf serum. For the isolation of cytoplasmic RNA, $\approx10^8$ cells were washed with phosphate-buffered saline, and then lysed by resuspending the cells in 1 ml of 0.5 M NaCl, 10 mM Tris (*p*H 7.5), 1.5 mM $MgCl_2$, 0.65% NP40. After incubation at 0 °C for 10 min, an equal volume of 7 M urea, 0.35 M NaCl, 10 mM Tris (*p*H 7.5), 10 mM EDTA, 1% SDS was added to the supernatant. This was extracted twice with phenol/chloroform/isoamyl alcohol (50:48:2) containing 0.1% 8-hydroxyquinoline, extracted twice with chloroform/isoamyl alcohol (24:1) and ethanol precipitated. For Northern blot analysis, 20 μg of cytoplasmic RNA was dissolved in sample buffer containing 1×running buffer (RB, 20 mM MOPS (*p*H 7.0), 1 mM EDTA, 5 mM NaAc), 50% (v/v) formamide, and 2.2 M formaldehyde. The RNA was then heated for 5 min at 65 °C and electrophoresed in 1–1.5% agarose gels containing 1×RB and 2.2 M formaldehyde. The position of the ribosomal RNA was visualized by acridine orange staining and the RNA was transferred to nitrocellulose paper in 20×SSC. The filters were air dried and baked for 2 h *in vacuo* at 80 °C. Hybridization conditions were as described elsewhere[23,24]. Plasmid probes used were labelled by nick-translation to a specific activity of $1\text{–}2\times10^8$ c.p.m. per μg.

Figure 1 is a Northern blot showing that the small RNAs homologous to 10λ5 are more abundant in the SV40-transformed cell line than in the parental NIH 3T3 cell line. The

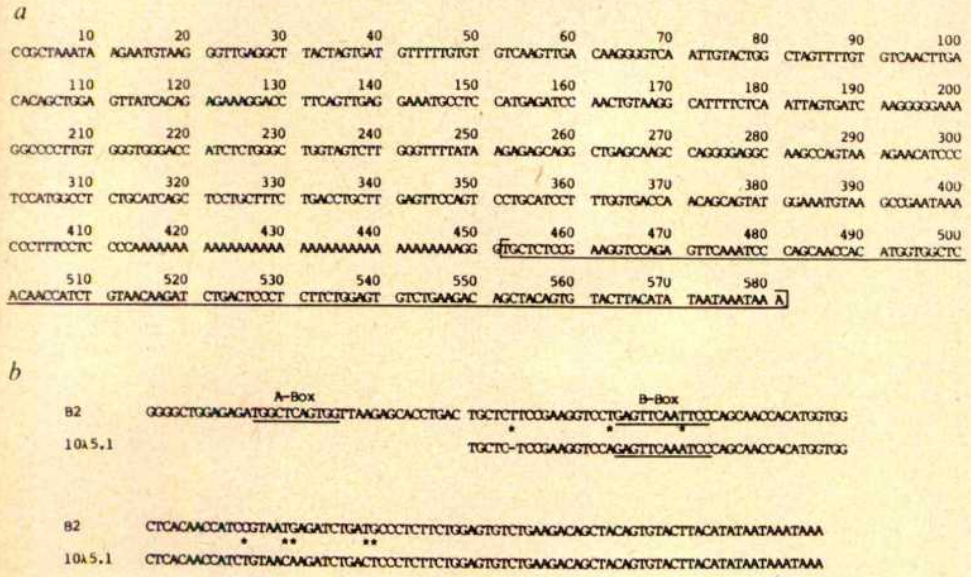


Fig. 2 *a*, The nucleotide sequence of 10λ5 determined by the chemical degradation procedure of Maxam and Gilbert[25]. The *B2* repeat sequences are underlined. The upstream sequence shows no extensive homology to any known sequence. *b*, Comparison of the sequence of the *B2* repeat in 10λ5 with the rodent *B2* consensus sequence[4]. Asterisks represent differences between the 10λ5 repeat and the *B2* consensus sequence. The sequences representing the A and the B boxes of the split polymerase III promoter[15] are underlined. The 10λ5 *B2* sequence lacks both the first 39 nucleotides at the 5′ end of the repeat, including the putative A box of the split polymerase III promoter, and 15–20 bp from the heterogeneous, A-rich 3′ end. However, over the remaining 131 bp, our sequence differs by only 6% from the consensus sequence.

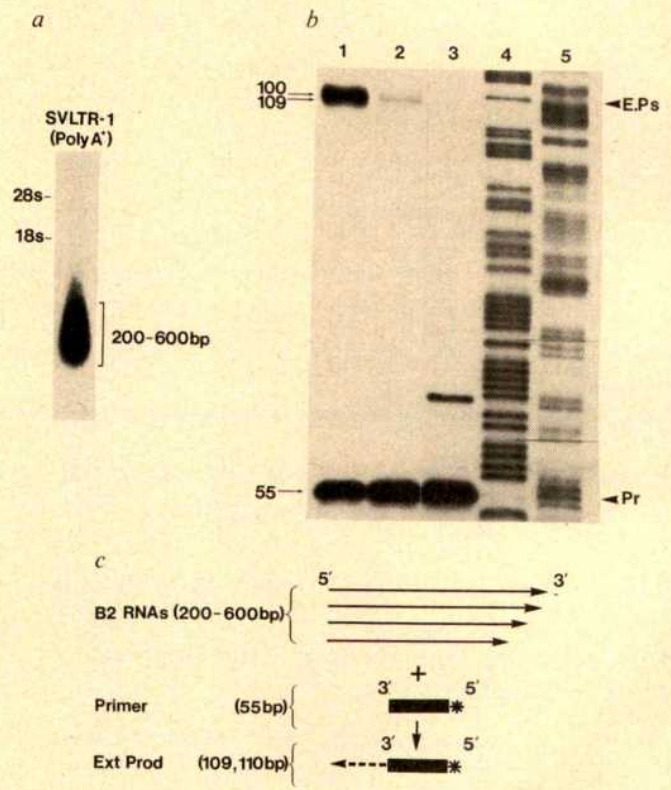


Fig. 3 *a*, Northern blot analysis of *B2* transcripts homologous to 10λ5 in 1 μg of poly(A)$^+$, cytoplasmic SVLTR-1 RNA. *b*, Primer extension analysis of SVLTR-1 cytoplasmic RNA using a *B2* gene specific probe. 1 μg (lane 1) and 100 ng (lane 2) of RNA were subjected to primer extension as described in *c*. Lane 3 is a mock reaction with no RNA. The extension products were sized alongside a sequencing ladder of known sequence. The *Bam*HI/*Hin*dIII fragment of the plasmid pXbs-73x201 (a *Xenopus borealis* 5S clone) was labelled at the *Bam* site using polynucleotide kinase and [γ^{32}P]ATP. The fragment was then subjected to Maxam–Gilbert sequencing reactions[25] for purines (lane 4) and pyrimidines (lane 5). The band which migrated just above the primer in lane 3 is double-stranded primer which remained undenatured. Pr denotes primer and E.Ps denotes extension products. *c*, A schematic illustration of the *B2* primer extension procedure. The 55-nucleotide *Bgl*II/*Ava*II coding strand fragment of 10λ5 (Fig. 2*a*, nucleotides 467–521), 5′ labelled at the *Bgl*II site, was prepared by standard procedures. This fragment was hybridized to total SVLTR-1 cytoplasmic RNA. The hybridized primer was then extended to the 5′ end of the RNA by reverse transcriptase and deoxynucleotide triphosphates (dNTPs) to generate two major extension products of 109 and 110 nucleotides. More than 80% of the extension products are associated with this doublet. The 3′ heterogeneity of *B2* RNAs is illustrated by the different-sized arrows.

Methods. Primer extension reactions were performed as described by McKnight *et al.*[26] with minor modifications. RNA samples were mixed with 5×10^4 c.p.m. (0.01–0.05 pmol) of single-stranded labelled DNA primer in 300 mM NaCl, 10 mM Tris *p*H 7.5 and 1 mM EDTA. The mixture was heated at 85 °C for 10 min, then annealed at 55 °C for 2.5 to 3.0 h. Following the hybridization, samples were incubated with 100 U ml^{-1} of avian myeloblastosis virus reverse transcriptase in a buffer containing 60 mM NaCl, 10 mM Tris *p*H 7.5, 10 mM dithiothreitol (DTT), 1 mM dNTPs, 8 mM $MgCl_2$, 50 μg ml^{-1} actinomycin D and 1,000 U ml^{-1} RNAsin. These reactions were incubated at 42 °C for 1 h and terminated by phenol extraction. The reactions were ethanol precipitated and resuspended in 90% formamide dye mix, heated at 90° for 3 min and loaded onto 8% polyacrylamide sequencing gels. Gels were autoradiographed for 12 h with a Cronex intensifying screen.

augmentation ranges over 5–20-fold depending on the growth state of the parental cells. These small RNAs are also detected by 10λ5 in Northern blots of poly(A)$^+$-selected RNAs (Fig. 3*a*). We find that these small RNAs are augmented in three other SV40-transformed mouse NIH 3T3 cell lines and in bovine papilloma virus- and methylcholanthrene-transformed cell lines (data not shown). Southern[5] hybridization analysis of genomic DNA from various mouse cell lines using 10λ5 as a probe always showed an intense smear pattern, indicating that 10λ5 contains a highly repetitive element.

The nucleotide sequence of 10λ5 is shown in Fig. 2*a*. The cDNA is 581 base pairs (bp) long, and at one end there is an incomplete copy of a member of the highly repetitive mouse *B2* family[3,4]. Family members are about 190 bp in size and are highly conserved in all rodent species. *B2* RNAs were initially observed to be present at higher levels in both Ehrlich carcinoma and MOPC cells than in mouse liver cells[6]. The reports of Scott *et al.*[1] and ourselves have now added significance to this initial observation by independently showing that these small RNAs are induced in various transformed mouse cells.

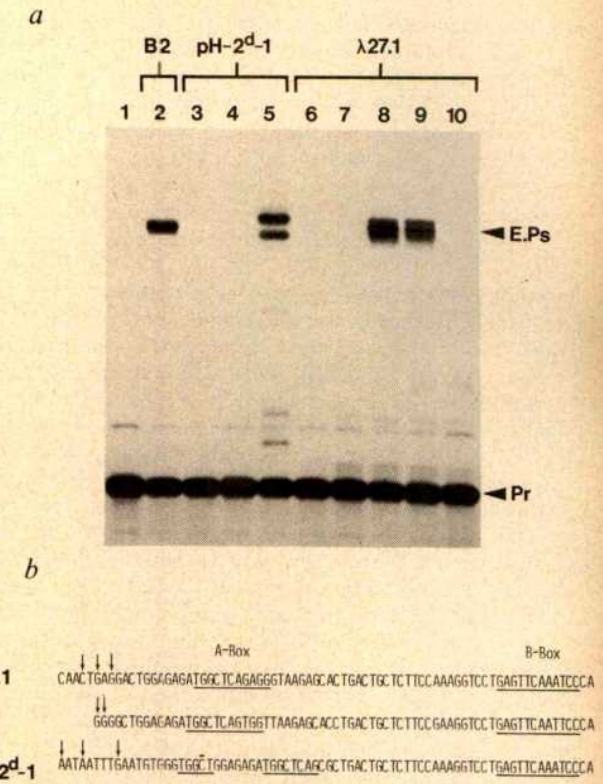


Fig. 4 *a*, Primer extension analysis of RNA transcribed *in vitro* with partially purified HeLa cell transcription factors and cloned DNAs containing *B2* elements. The two clones studied were pH-2^d-1 and λ27.1. The plasmid pH-2^d-1 contains a *B2* element in the 3′-untranslated region of the *H-2D* mouse class I gene of the major histocompatibility complex (MHC)[8] and the λ clone λ27.1 contains a mouse class I MHC pseudogene which has a *B2* element in the third intron[7]. Lane 1, a mock reaction with the primer alone; lane 2, the extension products for 500 ng of SVLTR-1 RNA; lanes 3–5, the extension products of RNAs generated by incubating pH-2^d-1 with polymerase III and TF-B (lane 3), polymerase III and TF-C (lane 4) and polymerase III, TF-B and TF-C (lane 5); lanes 6–10, the extension products of RNAs produced by incubating λ27.1 with polymerase III and TF-B (lane 6), polymerase III and TF-C (lane 7) polymerase III, TF-B and TF-C (lane 8), polymerase III, TF-B, TF-C and 2 μg ml^{-1} of α-amanitin (lane 9) and polymerase III, TF-B, TF-C and 200 μg ml^{-1} of α-amanitin (lane 10). It is not clear why the *in vivo* pattern is more homogeneous than the *in vitro* patterns. Longer exposures of this autoradiogram show that the *in vivo* starts as seen in lane 2 contain other minor bands within 2–3 bp both 5′ and 3′ to the starts shown. Pr denotes primer and E.Ps denotes extension products. *b*, A sequence comparison of the *B2* elements transcribed *in vitro* with the *B2* consensus sequence. Both pH-2^d-1 and 27.1 are aligned with the *B2* consensus sequence by their B box homologies. The A and B box sequence homologies are underlined and the *in vitro* and *in vivo* initiation sites are denoted by arrows.

Methods. A HeLa cell S100 extract was prepared as described by Weil *et al.*[27]. The transcription factor (TF) assays and the first step of the purification were modifications of those described by Segall *et al.*[16]. The extract was loaded onto a phosphocellulose column (P11 Whatman) pre-equilibrated in buffer A (20 mM HEPES *p*H 7.5, 20% glycerol, 0.2 mM EDTA and 0.5 mM DTT) and 100 mM KCl. TF-B activity was eluted with buffer A and a linear salt gradient of 100–350 mM KCl. Active fractions were pooled and dialysed for 8 h against buffer A and 50 mM KCl. The dialysate was loaded onto a DEAE-cellulose (DE-52 Whatman) column pre-equilibrated in the same buffer. The column was developed with buffer A and a linear salt gradient of 50–300 mM KCl. At this point, TF-B was 100-fold purified and contained <5% contaminating TF-C activity and no detectable TF-A activity. After the elution of TF-B, the phosphocellulose column was washed extensively with buffer A and 350 mM KCl. TF-C activity was then step eluted with buffer A and 600 mM KCl. Protein-containing fractions were pooled and dialysed for 8 h against buffer A and 200 mM KCl. The dialysate was loaded onto a native DNA cellulose column pre-equilibrated in the same buffer. The column was developed with buffer A and 1 M KCl. Protein-containing fractions were dialysed for 8 h against buffer A and 200 mM KCl. At this point, TF-C was purified 250-fold and contained < 5% contaminating TF-B and no detectable TF-A activity. Both TF-B and TF-C contained endogenous RNA polymerase III as measured by a nonspecific template assay[28]. The addition of purified RNA polymerase III had little effect on TF activity in the conditions used in our experiments. The standard transcription reactions were in a final vol. of 50 μl and contained 20 mM HEPES *p*H 7.5, 10% glycerol, 5 mM $MgCl_2$, 66 mM KCl, 0.5 mM DTT, 200 μM of rATP, CTP, GTP and UTP, 0.8 μg of plasmid or λ clone DNA, 2.5 μl TF-C and 12 μl TF-B. Incubations were carried out at 23 °C for 1 h and were terminated by phenol extraction. The reactions were ethanol precipitated and the pellet was resuspended in 10 mM Tris, 1 mM EDTA. The RNA was then assayed by primer extension analysis as described in Fig. 3 legend. Primer extension products were sized by electrophoresis alongside a sequencing ladder of known sequence.

In the cytoplasm, *B2* sequences are found mainly in RNAs of low relative molecular mass that are not associated with polysomes[6], but can also be present in larger, polysome-associated mRNAs at the 3′-untranslated end of the transcript[7–12]. The cloned *B2* repeat in 10λ5 appears part of a larger transcript, as there is 450 bp of sequence 5′ to the *B2* repeat. Figure 2*b* compares the 10λ5 *B2* sequence with the consensus *B2* sequence[4].

The homologies to the polymerase III promoter sequences[13] within the *B2* consensus sequence suggested that the small RNAs may be polymerase III transcripts. The heterogeneous nature of these RNAs could be a result of faithful initiation but irregular termination of transcription. Our examination of the sequence of a number of *B2* elements shows that only a subset contains an efficient polymerase III terminator—a cluster of four or more T residues surrounded by G+C-rich sequences[14]. Accordingly, one would then expect these cytoplasmic *B2* RNAs to have similar 5′ ends and these ends to map near the 5′ end of the element, because all polymerase III transcripts except for 5S RNA start 10–20 nucleotides upstream from the A box[15].

We tested this hypothesis using primer extension. As a primer we used a 55-bp *B2*-specific fragment from 10λ5. The 5′ end of the primer is located 31 bp downstream from the putative A box in the *B2* consensus sequence and 55 bp downstream from the 5′ end of the element (see Fig. 2*b*). We annealed this primer to total cytoplasmic RNA and extended the hybrids with reverse transcriptase as shown schematically in Fig. 3*c*. We find two major extension products 109 and 110 bp in size (Fig. 3*b*), which shows that the 5′ ends of the mature cytoplasmic *B2* RNAs are located 12 and 13 nucleotides upstream from the A box. This result strongly suggests that the small cytoplasmic *B2* RNAs are polymerase III transcripts.

To corroborate this conclusion, we used a partially purified polymerase III transcription system to transcribe two clones, pH-2^d-1 (ref. 8) and λ27.1 (ref. 7), which contained *B2* elements. Transcription of polymerase III genes with split promoters such as transfer RNA genes and the adenovirus virus-associated (VA) genes requires, in addition to polymerase III, transcription factors B and C (TF-B and TF-C)[16]. Primer extension analysis revealed that neither pH-2^d-1 or λ27.1 was an effective template for polymerase III unless the reaction mix also contained both TF-B and TF-C (Fig. 4*a*, lanes 3–5 for pH-2^d-1 and lanes 6–8 for λ27.1). The 5′ ends of the *B2* RNAs produced *in vitro* (Fig. 4*a*, lanes 5, 8) align closely with the 5′ ends of the *in vivo B2* RNAs (Fig. 4*a*, lane 2). Figure 4*b* summarizes these *in vitro* and *in vivo* start positions. Finally, λ27.1 was incubated with polymerase III, TF-B and TF-C in the presence of 2 μg ml^{-1} (Fig. 4*a*, lane 9) and 200 μg ml^{-1} (Fig. 4*a*, lane 10) of α-amanitin. The lower level inhibits polymerase II transcription while the higher level inhibits polymerase III[29]. Whereas 2 μg ml^{-1} of α-amanitin has no effect on *B2* transcription, 200 μg ml^{-1} is completely inhibitory.

The transcription factor requirements and the α-amanitin sensitivity indicate clearly that the mouse *B2* elements we have

studied are transcribed by polymerase III *in vitro*. In this respect, the mouse *B2* family is equivalent to various other *Alu*-like sequences known to be polymerase III templates *in vitro* (for review see ref. 17). Our primer extension analysis of cytoplasmic RNA shows that these repeats are also functioning as expressed polymerase III genes *in vivo*. *B2* RNA sequences can therefore arise as parts of polymerase II transcripts when the repeat is present within a polymerase II transcription unit, or they can be expressed independently by their own polymerase III promoters.

We have also studied 5S RNA gene expression and find no significant difference in the cytoplasmic levels of the 5S RNAs between the parental cell line and the SV40-transformed derivative (data not shown). Thus, there is no generalized induction of polymerase III transcription in SV40-transformed cells. Evidence for the regulation of specific polymerase III transcripts already exists for the expression of the oocyte-specific 5S genes in *Xenopus* (for review see ref. 18), and the brain-specific expression of the rat ID sequences[19,20]. Moreover, it has recently been shown that E1A induction of adenovirus VA RNAs is at least in part due to elevated levels of polymerase III transcription factor C (W. K. Hoeffler and R. G. Roeder, and S. Yoshinaga and A. Berk, personal communications). If the induction of the *B2* RNAs is at the level of transcription, this would suggest the presence of a polymerase III transcription factor(s) whose activity is sensitive to growth conditions and oncogenic transformation. It may be that the majority of the *B2* repeats, like similar highly repeated *Alu*-like sequences, have no useful function in the cell and have simply keyed themselves into a growth-sensitive polymerase III transcription system. Thus, other templates for this system could also exist and these may have physiologically more important roles for the rapidly dividing cell. It will therefore be interesting to study how other polymerase III transcripts such as the small 7SL RNA involved in secretion[21], tRNAs or 4.5S RNAs are affected by transformation.

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1. Scott, M. R. D., Westphal, K. & Rigby, P. W. J. *Cell* **34**, 557-567 (1983).
2. Schutzbank, T., Robinson, R., Oren, M. & Levine, A. J. *Cell* **30**, 481-490 (1982).
3. Kramerov, D. A., Grigoryan, A. A., Ryskov, A. P. & Georgiev, G. P. *Nucleic Acids Res.* **6**, 697-713 (1979).
4. Krayer, A. S. *et al. Nucleic Acids Res.* **10**, 7461-7475 (1982).
5. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
6. Kramerov, D. A., Lekakh, I. V., Samarina, O. P. & Ryskov, A. P. *Nucleic Acids Res.* **10**, 7477-7491 (1982).
7. Steinmetz, M. *et al. Cell* **25**, 683-692 (1981).
8. Lalanne, J. L. *et al. Nucleic Acids Res.* **10**, 1039-1049 (1982).
9. Reyer, A. A., Schold, M. & Wallace, R. B. *Immunogenetics* **16**, 1-9 (1982).
10. Moore, K. W. *et al. Science* **215**, 679-682 (1982).
11. Brickell, P. M. *et al. Nature* **306**, 756-760 (1983).
12. Ryskov, A. P., Ivanov, P. L., Kramerov, D. A. & Georgiev, G. P. *Nucleic Acids Res.* **11**, 6541-6558 (1983).
13. Traboni, C., Giliberto, G. & Cortese, R. *EMBO J.* **1**, 415-420 (1982).
14. Bogenhagen, D. F. & Brown, D. D. *Cell* **24**, 261-270 (1981).
15. Ciliberto, G. *et al. Cell* **32**, 725-733 (1983).
16. Segall, J., Matsui, T. & Roeder, R. G. *J. biol. Chem.* **255**, 11986-11991 (1980).
17. Jelinek, W. R. & Schmid, C. W. *A. Rev. Biochem.* **51**, 813-844 (1982).
18. Korn, L. J. *Nature* **295**, 101-105 (1982).
19. Sutcliffe, J., Milner, R. J., Bloom, F. E. & Lerner, R. A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4942-4946 (1982).
20. Sutcliffe, J., Milner, R. J., Gottesfeld, J. M. & Lerner, R. A. *Nature* **308**, 237-241 (1984).
21. Walter, P. & Blobel, G. *Nature* **299**, 691-698 (1982).
22. Kriegler, M. & Botchan, M. *Molec. cell Biol.* **3**, 325-339 (1983).
23. Wahl, G. M., Stern, M. & Stark, G. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683-3687 (1979).
24. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1982).
25. Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
26. McKnight, S. L., Davis, E. R., Kingsbury, R. & Axel, R. *Cell* **25**, 385-398 (1981).
27. Weil, P. A. *et al. J. biol. Chem.* **254**, 6163-6173 (1979).
28. Roeder, R. G. *J. biol. Chem.* **249**, 241-248 (1974).
29. Weinmann, R. & Roeder, R. G. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1790-1794 (1974).

ATP-dependent specific binding of Tn*3* transposase to Tn*3* inverted repeats

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Transposons are discrete segments of DNA which are capable of moving from one site in a genome to many different sites[1,2]. Tn*3* is a prokaryotic transposon which is 4,957 base pairs (bp) long and encodes a transposase protein which is essential for transposition[3–7]. We report here a simple method for purifying Tn*3* transposase and demonstrate that the transposase protein binds specifically to the ends of the Tn*3* transposon in an ATP-dependent manner. The transposase protein binds to linear double-stranded DNA both nonspecifically and specifically; the nonspecific DNA binding activity is sensitive to challenge with heparin. Site-specific DNA binding to the ends (inverted repeats) of Tn*3* is observed only when binding is performed in the presence of ATP; this ATP-dependent site-specific DNA binding activity is resistant to heparin challenge. Our results indicate that ATP qualitatively alters the DNA binding activity of the transposase protein so that the protein is able to bind specifically to the ends of the Tn*3* transposon.

DNA sequence analysis reveals that the Tn*3* transposase coding frame is 3,012 bp in length, which would allow for a protein of relative molecular mass (M_r) ~113,000 (113K). This size estimate agrees well with results obtained in experiments where Tn*3*-specific proteins have been identified by radioactive labelling in the minicell strain DS410 (refs 7–9). The purification of Tn*3* transposase was first reported by Fennewald *et al.*[10]. This protein exhibited a heat-sensitive nonspecific single- and double-stranded DNA binding activity. However, no additional enzymatic activities associated with the Tn*3* transposase protein have been reported. To learn more about the Tn*3* transposase, we devised a simpler purification scheme than that reported previously[10]. Our purification was facilitated by the observation that the wild-type transposase protein accumulates to ~1.5% of total cell protein when a plasmid bearing a Tn*3* repressor mutant (pMB8:Tn*3*$^{\circ}$5)[6], which transposes at a high frequency[6,11], is grown in minicell strain DS410[12,13] (Fig. 1).

Briefly, our method consists of four essential steps: membrane extraction, polyethyleneimine precipitation, step dialysis and separation by phosphocellulose chromotography. The method is simple, rapid, gentle and yields milligram quantities of transposase protein (see Fig. 2). The identity of the transposase protein was confirmed by amino-acid analysis and N-terminal sequence analysis performed at the Brookhaven protein sequencing facility at Brookhaven National Laboratories (unpublished data), and our results agree with those reported by Ditto *et al.*[14].

The transposase protein isolated in this manner binds nonspecifically to linear DNA fragments (Fig. 3); this DNA binding activity can be abolished by the addition of heparin, a mucopolysaccharide which competes with DNA for DNA binding sites. We used an assay essentially identical to that described by Wishart *et al.*[15]. The transposase protein is added together with DNA fragments in 250 μl of binding buffer (10 mM $MgCl_2$, 10 μg ml^{-1} bovine serum albumin, 100 mM KCl, 10 mM Tris-HCl *p*H 7.6, 0.1 mM dithiothreitol and 0.1 mM EDTA), ATP and heparin as described in Fig. 3 legend. The mixture is then incubated at 27 °C for 5 min and filtered through nitrocellulose filters. In our binding conditions, the transposase protein is retained quantitatively on the filters together with any DNA fragment to which the protein has bound, while unbound DNA fragments flow through. Filters are then washed extensively with binding buffer, and DNA fragments, retained on the filter by

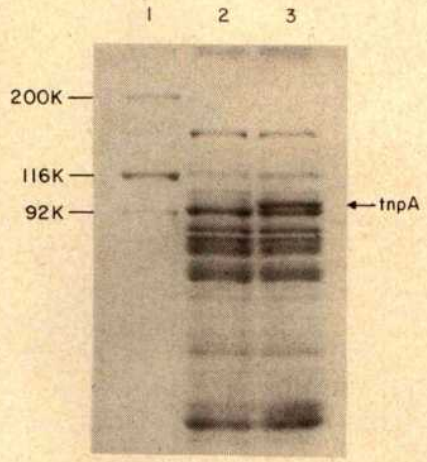


Fig. 1 Transposase protein accumulates in DS410 minicells. Lane 1 contains BioRad high-M_r markers as indicated. Proteins synthesized in DS410 cells containing pMB8:Tn*3* 72 (lane 2; transposase-negative)[6] or pMB:Tn*3* 5 (lane 3; transposase-positive)[6] were separated on a 10% polyacrylamide gel and stained with Coomassie brilliant blue. The transposase band (tnpA) is clearly visible.

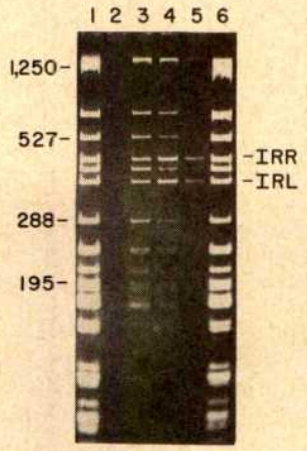


Fig. 3 Transposase binds to linear double-stranded DNA fragments. Lanes 1, 6, marker lanes of pMB::Tn*3* digested with *Hpa*II. Lanes 2–5, HpaII fragments retained on nitrocellulose filters in the presence, respectively of 0 μg transposase, 2.5 μg transposase, 2.5 μg transposase plus 8 mM ATP, and 2.5 μg transposase plus 10 μg ml^{-1} heparin with 8 mM ATP. Representative fragment sizes are indicated in base pairs. The fragments containing the Tn*3* left (IRL) and right (IRR) inverted repeats are also indicated.

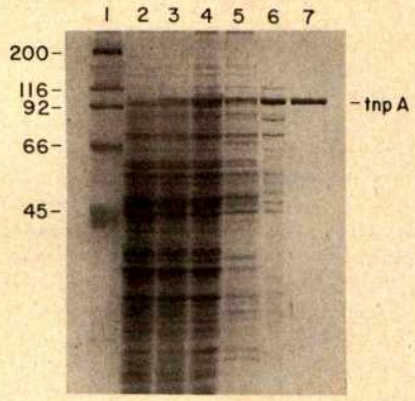


Fig. 2 Purification of the Tn*3* transposase. Protein samples obtained at various steps in the purification scheme were separated on a 10% polyacrylamide gel and stained with Coomassie brilliant blue. Lane 1, BioRad high-M_r protein markers ($\times 10^{-3}$) as indicated; lane 2, total cell lysate from transposase-negative control strain pMB:Tn*3* 72/DS410; lane 3, marker total cell lysate from transposase-overexpressing strain pMB:Tn*3* 5/DS410; lane 4, total cell lysate from transposase-overproducing strain pMB:Tn*3* 5/DS410 from which transposase was purified; lane 5, proteins extracted from membrane fraction with 2% Triton X-100; lane 6, nucleic acid binding proteins from the membrane fraction were precipitated by adding polyethyleneimine (final concentration 1.5%), the pellet was then extracted with 1 M ammonium sulphate, the extract was step-dialysed to remove ammonium sulphate, and proteins which precipitated in the 50–20 mM ammonium sulphate step were run in this lane; lane 7, transposase protein (4 μg) eluted from a phosphocellulose column via a linear gradient of 50 mM to 1 M potassium phosphate.

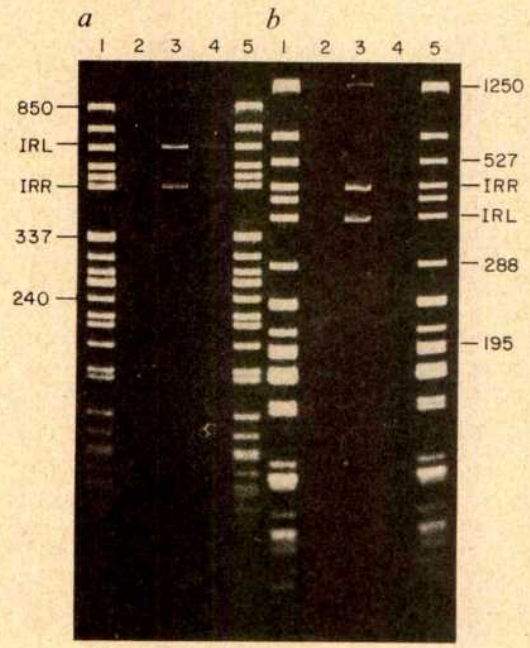


Fig. 4 Transposase site-specific DNA binding with DNA from *Cfo*I (*a*) and *Hpa*II (*b*) digestions of pMB8::Tn*3*; *a*, lanes 1, 5, *Cfo*I digestions of pMB8::Tn*3* lanes 2–4, *Cfo*I DNA fragments bound in the presence of 0 μg transposase, 2.5 μg transposase with 8 mM ATP, and 2.5 μg transposase with 16 mM ATP, respectively. *b*, Lanes 1, 5, *Hpa*II digestions of pMB8::Tn*3*, lanes 2–4, DNA fragments bound in conditions identical to those in *a*, lanes 2–4, respectively.

binding to the transposase protein, are eluted in 1 ml elution buffer (0.2% SDS, 30 mM Tris-HCl *p*H 7.6), ethanol-precipitated, and run on polyacrylamide gels next to marker digestions of the plasmid from which the original DNA (pMB:Tn*3* digested with *Hpa*II) was obtained. The results of these experiments indicate whether or not the transposase is able to bind to DNA and also provide information about binding specificity, as DNA fragments retained by the transposase protein can be compared directly with the original DNA fragments.

Because Tn*3* moves as a non-permuted element, that is, the linear arrangement of the transposon is unchanged during transposition[16], some factor must exist which is able to recognize the ends of the transposon as a first step in the transposition process. As the transposase is known[17] to be required for transposition of Tn*3* and as the transposase is known to act in *trans*[2,17], it is logical that such a site-specific binding activity capable of recognizing the ends of Tn*3* might reside in the transposase protein.

To determine whether or not the transposase possessed the ability to bind specifically to the ends of the Tn*3* transposon, we performed the above binding assay in a variety of conditions. Altering KCl, $MgCl_2$ and heparin concentrations affected the relative efficiency of binding, but no specific binding of DNA fragments containing the inverted repeats of Tn*3* was observed. Altering binding time or protein/DNA ratios also had no effect on binding specificity (data not shown).

Because some of the enzymes involved in DNA replication and recombination have been shown to use ATP as a free energy source[18,19], we added ATP to our reaction mixture to determine whether ATP might affect the DNA binding activity of the transposase protein. We found that ATP produced a dramatic change in DNA binding activity; in the presence of 8 mM ATP, the transposase protein was able to bind specifically to DNA fragments containing the ends of the Tn*3* transposon in a complex which was resistant to challenge with 10 $\mu g\ ml^{-1}$ heparin (Fig. 3, lanes 4, 5). In the absence of ATP, all DNA fragments could be removed from the transposase protein simply by adding heparin to 10 $\mu g\ ml^{-1}$, and no specific DNA binding was observed. In the presence of 8 mM ATP, fragments containing the 38-bp inverted terminal repeats of the Tn*3* transposon remained bound to the transposase protein even in the presence of 10 $\mu g\ ml^{-1}$ heparin (Fig. 3, lane 5; Fig. 4*b*, lanes 1-5).

The specificity of this binding activity was confirmed by repeating the experiment using pMB8:Tn*3* DNA digested with a different enzyme, *Cfo*I. Again, only the fragments containing the Tn*3* inverted repeats were retained on filters in the presence of ATP and heparin (Fig. 4*a*, lanes 1-5).

Our results suggest that ATP qualitatively alters the binding specificity of the transposase protein. However, one alternative explanation is that more than one DNA binding site exists on the transposase protein, only one or a few of which are capable of binding to the ends of the Tn*3* transposon. In this case, the ATP might not be required directly for recognition of the Tn*3* inverted repeats, but might merely decrease the nonspecific DNA binding activity relative to site-specific DNA binding activity. However, this seems not to be the case because, at a heparin concentration of 10 $\mu g\ ml^{-1}$, in the absence of ATP, all DNA fragments are competitively removed and no binding specificity is observed.

It is possible that ATP addition alters metal concentrations, causing the transposase protein to bind specifically to the inverted repeats of Tn*3*. Preliminary results from experiments in which residual magnesium was removed by the addition of EDTA to 25 mM indicate that magnesium is not essential for specific binding of the transposase protein, although it does seem to increase the efficiency with which the transposase protein binds to the DNA fragments containing an inverted repeat.

As transposons promote non-homologous genomic rearrangements, they are believed to be evolutionarily significant. Our finding that the transposase is capable of binding to the ends of the Tn*3* transposon and that this binding is specifically affected by ATP is exciting in that it suggests that ATP might be fuelling at least the initial step in the transposition mechanism (that is, recognition of the ends of the transposon by the transposase). We are now attempting to analyse the nature of this effect of ATP on binding specificity in greater detail. We are also investigating other transposition-related activities which current models[20-22] for the molecular mechanism of Tn*3* transposition would predict to be associated with an active transposase or transposase complex.

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1. Calos, M. P. & Miller, J. H. *Cell* **20**, 579-595 (1980).
2. Kleckner, N. *A. Rev. Genet.* **15**, 341-404 (1981).
3. Heffron, F. in *Mobile Genetic Elements* (ed. Shapiro, J.) 223-260 (Academic, New York, 1983).
4. Heffron, F., Bedinger, P., Champoux, J. J. & Falkow, S. *Proc. natn. Acad. Sci. U.S.A.* **74**, 702-706 (1977).
5. Gill, R., Heffron, F., Dougan, G. & Falkow, S. *J. Bact.* **136**, 742-756 (1978).
6. Heffron, F., McCarthy, B. J., Ohtsubo, H. & Ohtsubo, E. *Cell* **18**, 1153-1163 (1979).
7. Gill, R. E., Heffron, F. & Falkow, S. *Nature* **282**, 797-801 (1979).
8. Chou, J., Lemaux, P. G., Casadaban, M. J. & Cohen, S. N. *Nature* **282**, 801-806 (1979).
9. Dougan, G., Saul, M., Twigg, A., Gill, R. & Sherratt, D. *J. Bact.* **138**, 48-54 (1979).
10. Fennewald, M. A., Gerrard, S. P., Chou, J., Casadaban, M. J. & Cozzarelli, N. R. *J. biol. Chem.* **256**, 4687-4690 (1981).
11. McCormick, M., Wishart, W. L., Ohtsubo, H., Heffron, F. & Ohtsubo, E. *Gene* **15**, 103-118 (1981).
12. Adler, H. I., Fisher, W. C., Cohen, A. & Hardigree, A. A. *Proc. natn. Acad. Sci. U.S.A.* **57**, 321-326 (1966).
13. Roozen, K. J., Fenwick, R. G. Jr & Curtis, R. III *J. Bact.* **107**, 21-33 (1971).
14. Ditto, M. D. *et al. J. Bact.* **149**, 407-410 (1982).
15. Wishart, W. L., Machida, C., Ohtsubo, H. & Ohtsubo, E. *Gene* **24**, 99-103 (1983).
16. Rubens, C., Heffron, F. & Falkow, S. *J. Bact.* **128**, 425-434 (1976).
17. Gill, R., Heffron, F., Dougan, G. & Falkow, S. *J. Bact.* **136**, 742-756 (1978).
18. Weinstock, G. M., McEntee, K. & Lehman, I. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 126-130 (1979).
19. Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, N. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3872-3876 (1976).
20. Grindley, N. D. F. & Sherratt, D. *Cold Spring Harb. Symp. quant. Biol.* **43**, 1257-1261 (1979).
21. Shapiro, J. A. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1933-1937 (1979).
22. Galas, D. J. & Chandler, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4858-4862 (1981).

Domains of action of *bithorax* genes in *Drosophila* central nervous system

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The diversification of all *Drosophila* segments posterior to the mesothorax is controlled by a single gene cluster, the bithorax complex (BX-C)[1]. Various observations have led to the conclusion that the BX-C is involved in the segmental determination of both the central nervous system (CNS) and the epidermis[1-6]. In general, the neural and epidermal effects of a given BX-C mutation were found to occur in the same segment. However, only one neural structure was examined in each of these experiments; furthermore, the segmental origin of these few structures was in most cases unclear, making it impossible to decide whether or not the action of the BX-C genes is confined to the same regions in the epidermis and the CNS. Here we describe a set of 18 uniquely recognizable fascicles in the adult CNS, and establish the segmental origin of each of them. We analyse the effect of different BX-C mutations on these fascicles, and show that each mutation affects a region that overlaps two consecutive segments. We conclude that in the CNS, the spatial domains of action of BX-C genes are segment-length units which are out of register with the epidermal segments.

The problem of defining the spatial domains of action of BX-C genes in the adult CNS is complicated by two facts. First, there has been a tendency towards the condensation of the CNS during insect evolution. Whereas in primitive insects the segmental ganglia are clearly separated, in flies the thoracic and abdominal ganglia fuse to form a single ventral ganglion with no detectable intersegmental boundaries. Second, very few segment-specific structures have been identified in the CNS of *Drosophila*, and therefore the description of the segmental transformations associated with a given BX-C mutation is severely limited. We have overcome both difficulties by studying the pattern of transversal commissures in the CNS of adult flies, using a monoclonal antibody (5D12, isolated by Y. N. Jan and L. Y. Jan) which labels a set of transversal fascicles at all developmental stages (Fig. 1). Other labelled structures are also present in the adult CNS (Fig. 1*d*-*f*), but will not be dealt with here. Early during neurogenesis, two transversal commissures, one anterior and one posterior, are formed within each segmental ganglion, both in *Drosophila*[7] and in the grasshopper[8] (Fig. 1*a*). In the grasshopper[8,9] and probably also in *Drosophila*[10], these two commissures are pioneered by neurones belonging to the same segmental ganglion. By the end of embryonic development, a number of different fascicles can be distinguished in each of these two commissures by using antibody

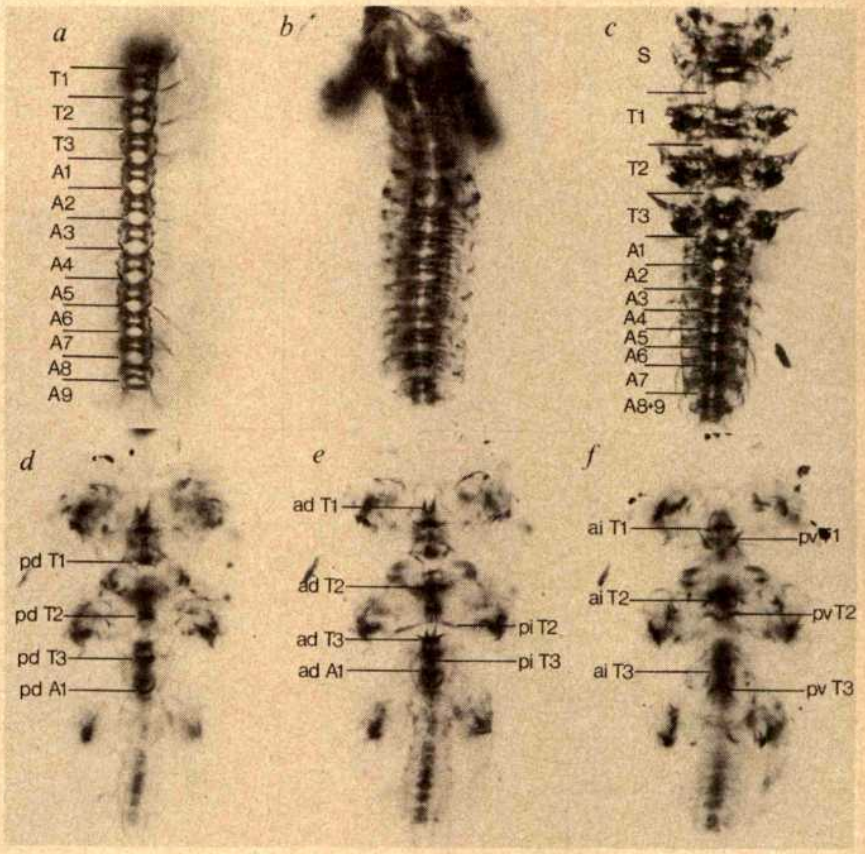


Fig. 1 The development of transversal fascicles revealed by antibody 5D12. *a*, CNS of a 12-h-old embryo. One anterior and one posterior commissure are seen in the three thoracic (T1–T3) and eight abdominal (A1–A8) segments. The ninth abdominal ganglion (A9) contains only one commissure. The three suboesophageal ganglia and the brain stems are out of focus. *b*, CNS of an early first-instar larva. Various distinct fascicles, only some of which are in focus in this photograph, can be distinguished within each commissure. *c*, CNS of a 9-h-old pupa. The major changes that will lead to the adult pattern have only just begun and the suboesophageal ganglia are still closely apposed to the thoracic ganglia. The organization of the fascicles in two clusters, one anterior and one posterior, can still be detected. *d*, Adult CNS, dorsal focal plane. *e*, Adult CNS, intermediate focal plane. *f*, Adult CNS, ventral focal plane. Most of the adult fascicles studied in the mutants can be detected, although some are slightly out of focus (compare with Fig. 2, bottom row). Abbreviations: ad, anterior dorsal fascicle; ai, anterior intermediate fascicle; av, anterior ventral fascicle; pd, posterior dorsal fascicle; pi, posterior intermediate fascicle; pv, posterior ventral fascicle.

Method. In all cases, the staining was done on formaldehyde-fixed nervous systems except for *a*, where heptane/formaldehyde-fixed whole embryos were used. The dissection, fixation and rinse were performed in phosphate buffer 0.1 M, *p*H 7.6. All the following steps were each performed for 6 h in the same buffer containing 0.3% deoxycholate and 0.3% Triton (PDT buffer): The samples were incubated in antibody 5D12 (1:10 in PTD containing 10% of normal goat serum), rinsed in PDT, incubated with biotinylated secondary antibody (1:200 in PDT), rinsed in PDT, incubated in avidin biotinylated peroxidase (1:1:100 in PDT) and rinsed in PDT. For larval ganglia, the incubation times were reduced to 3 h instead of 6 h. All secondary immunoreagents were from Vector Labs. The samples were then processed for peroxidase staining, dehydrated, cleared and mounted. The adult ganglion was understained for photograph purposes.

5D12 (Fig. 1*b*). Little change is observed in this pattern up to the early pupal stage (Fig. 1*c*), but during the next 2 days most of the abdominal fasicles disappear and the thoracic fascicles undergo major modifications such that the segmental origins of the adult structures become less clear (Fig. 1*d*–*f*). However, by studying intermediate pupal stages, we have been able to follow the fate of a given fascicle throughout metamorphosis (Fig. 2) and have traced all the transversal fascicles that we detect in the adult CNS to the anterior or posterior embryonic commissure of a particular segment.

To simplify the description of mutant phenotypes, we have assigned a symbol to each adult fascicle within a given segment. The symbol summarizes the origin of the fascicle ('a' or 'p' for fascicles derived from the anterior and posterior embryonic commissures) and its position within the adult ganglion ('v', 'i' or 'd' for ventral, intermediate or dorsal). Some fascicles revealed by antibody 5D12 still show a clear homology from segment to segment in the adult, and will not be considered further. However, most of them have assumed segment-specific shapes in the adult (for example, compare the ad fascicles in segments T1, T2, T3 and A1; Fig. 2) thereby enabling us to determine the effect of BX-C mutations on this process of segmental diversification.

We have examined how this unique pattern of fascicles is affected by mutations in three consecutive regions of the BX-C: the *Ubx*, *bxd* and *iab-2* regions (Table 1, Fig. 3).

All recessive mutations in the *Ubx* region[11] produce transformation of the anterior metathorax (T3) into an anterior mesothorax (T2) in the epidermis. Two classes of mutations, *bx* and *abx*, have been distinguished on the basis of their localization, partial complementation and minute differences in

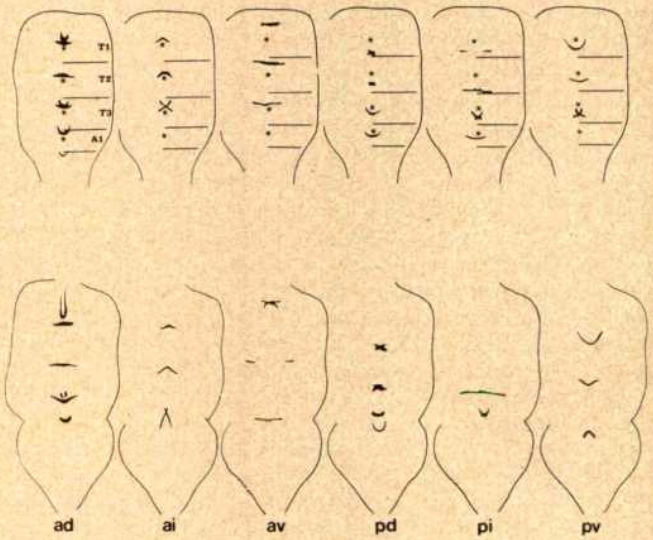


Fig. 2 Metamorphosis of the CNS. The transformation of the larval pattern into the adult pattern was followed at 9, 15, 20, 24, 48 and 73 h after the onset of metamorphosis. The first line of pictures are camera lucida drawings of a CNS during early metamorphosis (15 h APF); the second line shows drawings of the adult pattern. Each row indicates the segmental homologues for one of the fascicles studied in this paper. In the pupal CNS, the large gap between commissures belonging to consecutive segments is marked by a line; the smaller gap between the anterior and posterior commissures within a segment is marked by an asterisk. Abbreviations as in Fig. 1.

Table 1 Effect of BX-C mutations on adult *Drosophila* fascicles

	T2						T3						A1						A2					
	a d	a i	a v	p d	p i	p v	a d	a i	a v	p d	p i	p v	a d	a i	a v	p d	p i	p v	a d	a i	a v	p d	p i	p v
bx^3																								
bx^3/Df																								
bx^{34e}							(±)										+		+			+		
abx^1					+		+		+	+														
abx^1/Df					+		+		+	+							+		+			+		
abx^2/Df					+		+		+	+	(±)						+		+			+		
bxd										(±)	+	+	+	+	+	+								
bxd/Df										(±)	+	+	+	+	+	+	+		+			+		
$Df/+$																	+		+			+		
$iab\text{-}2$																	+		+			+		
pbx																								
pbx/Df										(±)	+		+	+			+		+			+		
bxd^1/Df										(±)	+		+				+		+			+		

Genotype	No. of nervous systems analysed
bx^3:	
bx^3/bx^3	9
$bx^3/Df(3R)bxd^{100}$	4
bx^3/Df:	
$bx^3/Df(3R)Ubx^{109}$	8
bx^{34e}:	
$bx^{34e}/Df(3R)bxd^{100}$	7
abx^1:	
abx^1/abx^1	7
$abx^1/Df(3R)bxd^{100}$	8
abx^1/Df:	
$abx^1/Df(3R)Ubx^{109}$	13
abx^2/Df:	
$abx^2/Df(3R)Ubx^{109}$	10
bxd:	
bxd^{100}/bxd^{125}	11

Genotype	No. of nervous systems analysed
bxd/Df:	
$bxd^{100}/Df(3R)Ubx^{109}$	7
$Df/+$:	
$Df(3R)Ubx^{109}/+$	8
$Df(3R)P10/+$	6
$iab\text{-}2/+$:	
$T(2;3)P10/+$	18
pbx:	
pbx^1/pbx^1	7
pbx^1/pbx^2	5
$pbx^1/Df(3R)100$	7
pbx/Df:	
$pbx^1/Df(3R)P10$	5
$pbx^1/Df(3R)Ubx^{109}$	8
bxd^1/Df:	
$bxd^1/Df(3R)Ubx^{109}$	8

The extent and frequency of the transformations are represented as follows. + Represents a rather complete transformation, +/− represents a partial transformation. In the latter case, either a fascicle is of an intermediate type or more often a normal and a transformed fascicle coexist. In one case (ai A1 in *pbx/Df*), +/− represents an additional fascicle that resembles not the adult ai T3, but its early pupal stage (see Fig. 2). Bold type indicates that the effect was seen in all mutants, normal type indicates an effect observed in a majority of the cases, and parentheses indicate an occasional effect. The results obtained with different genotypes were pooled whenever the results appeared to be identical. The lower half of the table shows the genotypes and numbers of nervous systems analysed.

phenotype[12]. We examined four mutations in this *Ubx* region: two *bx* alleles, bx^3 and bx^{34e}, and two *abx* alleles, abx^1 and abx^2. None of the *bx* mutations gave any detectable effect on any fascicle, other than an occasional slight malformation of ad T3 in bx^{34e} hemizygous flies. This is perhaps not surprising in view of the fact that *bx* mutations, and in particular bx^3, have been reported to have no effect on sensory neurones[13,14], and at most a minimal effect on the CNS[15,16]. On the other hand, both *abx* alleles have a marked effect on one mesothoracic fascicle (pi T2) and on three metathoracic fascicles (ad, av and pd T3). These effects are detected in homozygous as well as hemizygous flies. In addition, a slight effect on pi T3 can be detected in some hemizygous abx^2 flies.

Two classes of recessive mutations are known in the *bxd* region[11]; *bxd* mutations transform the anterior first abdominal segment (A1) into an anterior T3, and to a lesser extent the posterior T3 into a posterior T2, while *pbx* mutations show only the latter transformation[17]. We examined four mutants in the *bxd–pbx* region: two extreme *bxd* alleles, bxd^{100} and bxd^{125}, and the two existing *pbx* alleles, pbx^1 and pbx^2. Both *bxd* mutations had a marked effect on two metathoracic fascicles (pi and pv T3) and on four first abdominal fascicles (ad, ai, av and pd A1), while a weaker effect was occasionally observed on pd T3. These effects were observed in heterozygous bxd^{100}/bxd^{125} as well as in hemizygous flies. The two *pbx* alleles showed essentially no phenotype when homozygous or when combined with a deficiency for the *Ubx* region (and part of the *bxd* region), $Df(3R)bxd^{100}$. However, they caused clear transformations when combined with a larger deficiency deleting the entire *Ubx–bxd* region (and part of the *iab-2* region), *Df(3R)P10*. In this combination, *pbx* also affected both metathoracic (pi T3, and occasionally pd t3) and first abdominal (ad A1, and to a lesser extent ai A1) fascicles.

Extreme *iab-2* mutations, like *TP10*, are embryonic lethal when homozygous. Whether or not this boundary coincides with a compartment boundary cannot be decided, since it is not known if compartments exist in the CNS. However, the analysis of mutant embryos revealed that the anterior part of the second abdominal segment (A2) is transformed into A1 (ref. 18). In the CNS, however, transformations were observed even in *TP*10/+ heterozygotes, indicating haplo-insufficiency for $iab\text{-}2^+$. In this case, the mutant phenotype does not lie in the transformation of existing fascicles, but in the persistence of abdominal fascicles that are normally suppressed during metamorphosis. Here again, the effect is seen in two consecutive segments; an additional pi fascicle is usually present in A1, and additional ad and pd fascicles are often observed in A2.

We conclude that the defects corresponding respectively to the *abx*, *bxd* and *iab-2* mutations define three consecutive regions in the CNS; their boundaries do not coincide with segmental boundaries, as in each case fascicles belonging to two different segments were affected. The linear order of these regions is entirely consistent with the observation made by Lewis[1] that consecutive genes in the BX-C are required in consecutive regions of the body, and indicates that the expression of the BX-C largely follows the same sequential

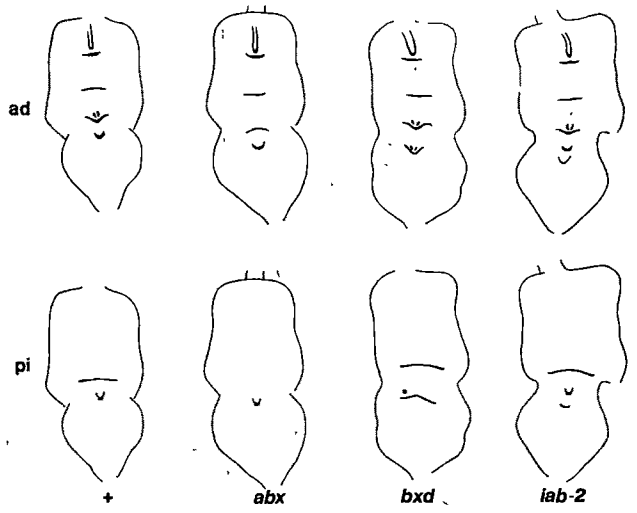


Fig. 3 Effect of BX-C mutants on the adult fascicles. The transformations undergone by two fascicles, an anterior one (ad) and a posterior one (pi), are illustrated for mutations in each of the three regions examined. *abx*: $abx^1/Df(3R)bxd^{100}$ flies; *bxd*: bxd^{100}/bxd^{125}; *iab-2*: *T(2;3)P10/+*. In all cases, the modification results in a fascicle resembling that of the anterior segment. Thus, in some cases the shape of the fascicle is changed (for example pi T3 in bxd); in some cases the fascicle disappears (for example pi T2 in *abx*); and in some an additional fascicle is formed (for example, pi A1 in *iab-2*). All figures are camera lucida drawings; for each genotype, we used a ganglion which showed a complete transformation; see Table 1 for the frequency of the different transformations in each genotype.

strategy in the epidermis and the CNS. The fact that the boundaries of these regions do not coincide with segmental boundaries clearly supports the 'frameshift' hypothesis proposed by Lewis[19] that the posterior regions of segments 'appear, in the presence of the mutants, to behave as though they were embryologically related to the anterior portion of the segment that follows, rather than precedes, them'. According to this hypothesis, the genes of the BX-C would act on segment-length units which include the posterior portion of one segment and the anterior portion of the following segment, and therefore are out of register with the segments themselves. In the case of the Ubx^+ gene analysis of the distribution of Ubx proteins in the CNS has provided a direct confirmation of this hypothesis[20,21].

The epidermal segments consist of two compartments, one anterior and one posterior[22]. It has been hypothesized that compartments rather than segments are the basic units of development[23], and that the limits of the domains of action of BX-C genes coincide with compartment boundaries[22,24,25]. Struhl[24] has recently rephrased the frameshift hypothesis in terms of compartments on the basis of his and other[26] results. Thus, the realm of action of BX-C genes would extend from an antero-posterior compartment boundary to the next one, and therefore include the posterior compartment of one segment and the anterior compartment of the following segment.

In the CNS, the three domains of action defined respectively by the *abx*, *bxd* and *iab-2* mutations extend from a posterior fascicle (pi) to another posterior fascicle (pd Table 1), implying that the limits of the domains are located in the posterior commissure. The observation that pd T3 is occasionally affected in *bxd* mutants suggests that pd may be at the very boundary between consecutive domains. Whether or not this boundary coincides with a compartment boundary cannot be decided, since it is not known if compartments exist in the CNS. However, the analysis of mutant phenotypes indicates that each transformation affects an entire segment-length unit. This result is particularly interesting in the phase of the *pbx* and *bxd* mutations, which in the adult epidermis affect predominantly the posterior T3 and anterior A1, respectively[1,17,27]. Our results revealed no clear difference in the domain of action of *pbx* and *bxd*. Thus, in different *pbx* combinations, whenever a transformation was detected, it affected fascicles on both sides of the T3–A1 segmental boundary. Similarly, when we reduced the *bxd* phenotype by using leaky *bxd* mutations the transformations in T3 and A1 were affected equally (Table 1). This indicates that a compartmental specificity of BX-C mutations similar to that observed in the epidermis is not readily detectable in the CNS. Thus, if compartments exist in the CNS, they do not seem to be important in the pattern of expression of BX-C genes.

We conclude that in the CNS, BX-C mutations affect entire segment-length units, suggesting that the domains of action of BX-C genes define 'genetic segments', similar to the morphological segments in their size but not in their boundaries. We emphasize, however, that our conclusions hold only for neurones involved in the diversification of the transversal fascicles labelled by 5D12; other structures may reveal a different pattern.

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1. Lewis, E B *Nature* **276**, 565–570 (1978)
2 Jimenez, F. & Campos-Ortega, J. A *Wilhelm Roux Arch. ges Physiol.* **190**, 370–373 (1981)
3 Green, S H *Nature* **292**, 152–154 (1981)
4 Teugels, E. & Ghysen, A. *Nature* **304**, 440–442 (1983)
5 Thomas, J. & Wyman, R. *Devl Biol* **102**, 531–533 (1984).
6 Ghysen, A , Jan, L Y. & Jan, Y. N *Cell* (in the press).
7 Jan, L Y & Jan, Y. N *Proc. natn Acad Sci. USA.* **79**, 2700–2704 (1982).
8 Goodman, C S , Bate, M. & Spitzer, N. *J. Neurosci.* **1**, 94–102 (1981).
9. Raper, J A., Bastiani, M. & Goodman, C S. *J. Neurosci.* **3**, 31–41 (1983)
10. Thomas, J B , Bastiani, M J , Bate, M & Goodman, C. S *Nature* **310**, 203–207 (1984).
11. Bender, W. *et al. Science* **221**, 23–29 (1983)
12 Lewis, E. B in *Developmental Biology Using Purified Genes* (eds Brown, D D & Fox, C F.,) 189–208 (Academic, New York, 1981).
13 Palka, J & Schubiger, M in *Development and Neurobiology of* Drosophila (eds Siddiqi, O., Babu, P , Hall, L M & Hall, J C.) 223–246 (Plenum, New York, 1980)
14 Ghysen, A , Janson, R. & Santamaria, P. *Devl Biol.* **99**, 7–26 (1983).
15 Ghysen, A *Nature* **274**, 869–872 (1978)
16. Strausfeld, N. J & Singh, R. N in *Development and Neurobiology of* Drosophila (Eds Siddiqi, O , Babu, P , Hall, L M & Hall, J. C) 267–290 (Plenum, New York, 1980)
17 Lewis, E. B *Am. Nat* **84**, 73–89 (1955)
18 Morata, G., Botas, J , Kerridge, S. & Struhl, G *J. Embryol. exp Morph* **78**, 319–341 (1983)
19. Lewis, E B *Cold Spring Harb Symp quant Biol.* **16**, 159–174 (1951)
20. White, R. A. H. & Wilcox, M. *Cell* **39**, 163–171 (1984)
21 Beachy, P. A., Helfand, S L & Hogness, D S *Nature* **314**, 545–551 (1985)
22 Garcia-Bellido, A , Ripoll, P & Morata, G *Nature new Biol.* **245**, 251–253 (1973)
23 Crick, F H C. & Lawrence, P *Science* **189**, 340–347 (1975)
24 Struhl, G. *Nature* **308**, 454–457 (1984)
25 Lawrence, P & Morata, G. *Cell* **35**, 595–601 (1983)
26 Morata, G & Kerridge, S *Nature* **290**, 778–781 (1981).
27. Minana, F J & Garcia-Bellido, A. *Wilhelm Roux Arch. ges Physiol.* **191**, 331–334 (1982)

Erratum

Change in motion of Pacific plate at 5 Myr BP

A. Cox & D. Engebretson
Nature **313**, 472–474 (1985)

ON page 472, the last line of the fourth paragraph was deleted. The second sentence should read: "In offshore basins in central California, there is further tectonic evidence for a component of plate convergence across the San Andreas fault beginning at ~5.5 Myr[4]."

This should be followed by a new paragraph beginning: "Along the Pacific/Juan de Fuca (PA-JF) spreading centre, two major shifts in rotation poles occurred..."

Model for Grampian tract evolution

DEWEY and Shackleton's stimulating and provocative model for the evolution of the Grampian tract of the Caledonide-Appalachian orogen[1] postulates subduction towards the south-east between about 510 Myr BP and at least 470 Myr BP. As a result, they reject previous suggestions that the extensive layered mafic and ultramafic intrusions of Connemara and north-east Scotland were emplaced (at 485–500 Myr BP) above a north-west-dipping subduction zone and formed the roots of Andean-type volcanic arcs[2] or the lower parts of batholiths, subsequently removed during Upper Ordovician erosion[3]. The more mafic of these plutons contain cumulus assemblages of the minerals olivine plus chromite, followed by magnesian ortho- and clinopyroxene, very calcic plagioclase, and interstitial primary amphibole—inferred to precipitate at deep-crust levels within island arcs from subduction-related, silica-saturated hydrous magnesian magmas[4,5].

Figure 1 shows normalized incompatible trace elements in samples of each complex that have essentially escaped both the interaction with upper-crust metasediments and the Grampian deformation and metamorphism that affects most of them. Subduction-related basalts from Japan and Vanuatu are also plotted in Fig. 1. The essential similarities of many inter-element ratios between these modern circum-Pacific island-arc lavas and the Ordovician plutons are clear. As a group, the trace-element patterns (Fig. 1) of these samples are characteristic of subduction-related magmas worldwide and are quite different from those erupted in other tectonic environments, such as strike-slip pull-aparts, which Dewey and Shackleton suggest as an alternative. A Recent basanite from Dish Hill, Southern California (Fig. 1) is a typical example of the predominant type of basic magmatism in the south-west USA, extruded since the inception of a strike-slip plate boundary in this region.

Dewey and Shackleton have commented[1] that their "model is invalid, at least for the British Isles", if these Lower Ordovician plutons are subduction-related. I agree that more research is needed, but nevertheless, emphasize that there is already sufficient knowledge of the geochemistry of Lower Ordovician magmatism in the Caledonian–Appalachian orogen that the data from this approach cannot be ignored when constructing overall models.

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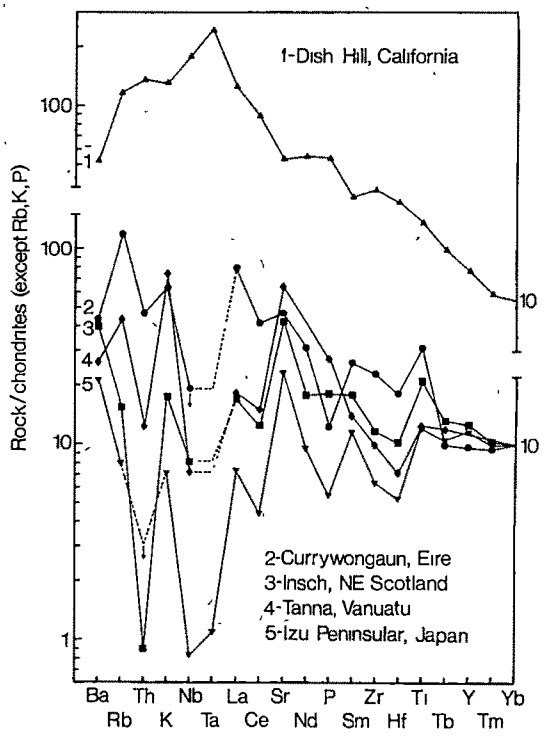


Fig. 1 Normalized[3] trace-element plot. Small arrows indicate element abundances below detection limits. The log scale represents: Abundance (mass) of each element in rock/Abundance in chondrites. Data are from refs 3 and 6.

1 Dewey, J F & Shackleton, R M *Nature* **312**, 115–121 (1984).
2 Yardley, B W D, Vine, F J & Baldwin, C. T *J geol Soc. Lond* **139**, 457–463 (1982)
3 Thompson, R. N, Morrison, M. A, Hendry, G L & Parry, S J *Phil. Trans R. Soc.* A310, 549–590 (1984)
4 Leake, B E *Geol. J spec. Publ* **10**, 221–248 (1978).
5 Perfit, M R., Gust, D A, Bence, A E, Arculus, R J & Taylor, S R *Chem Geol.* **30**, 227–256 (1980).
6. Dupuy, C *et al Earth planet Sci Lett.* **60**, 207–225 (1982)

DEWEY AND SHACKLETON REPLY—We thank Thompson for his geochemical data and conclusions and agree that a successful model for the evolution of the Grampian tract must account for geochemical and petrological data. Clearly, from the perspective of incompatible element pattern recognition, the Connemara and Banffshire intrusions are similar to the magmatic rocks of some modern arcs, but our lack of geochemical expertise disqualifies us from commenting on that data. However, we note that calc-alkaline magmatism occurs in a wide variety of non-arc settings including collisional environments such as eastern Anatolia. An important mechanism in collisional orogens is lithospheric delamination[1], which allows hot fertile mantle to gain access to levels as high as the base of a thickened crust. This causes rapid uplift and denudation and a surge of mafic magmatism and possibly partial melting of a mafic lower crust. We suggest that such a mechanism was responsible for the Grampian mafic intrusions. Our use of the term 'pull-apart' is simply kinematic and does not imply a particular regional tectonic setting. Pull-aparts in strike-slip systems occur in almost every type of regional tectonic environment including island arcs. In collisional systems, they may localize calc-alkaline magmatic rocks (for example, Erzinçan basin on the North Anatolian Fault). A particular problem with the Connemara intrusions is that we know little of their original form and intrusive mechanism, though they appear to have a regionally disrupted sheet-like form. A particular problem in considering the intrusions as arc roots is that they were intruded into a pile of nappes during the latest stages of shortening. A West Pacific model, therefore, seems inappropriate because most of the West Pacific is in deviatoric tension where shortening is rare.

Lastly, the model is developed largely from data for Newfoundland where it seems difficult to refute, and then extrapolated to the British Isles. It is quite certain that the obduction model in its simplest form cannot account for the Taconian events of the Appalachians south of Quebec, because the tell-tale mafic detritus, particularly chromite, is virtually absent from the Taconic flysch belt in the US Appalachians. Hence, although it is also possible that the obduction model is inappropriate for the early Caledonides and is unique to the Canadian Appalachians, we cannot yet think of a better model.

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1 Bird, P *J geophys Res* **83**, 4975–4987 (1978)

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Trading places

Duncan Davies

Research and Innovation: A Record of the Wolfson Technological Projects Scheme 1968 - 1981.
By L. Rotherham.
Clarendon: 1984. Pp.99. £12.50, $18.95.
Foresight in Science: Picking the Winners. By John Irvine and Ben R. Martin.
Frances Pinter, London/Longwood, Dover, NH: 1984. Pp.166. £12.50, $21.

THOSE choosing the investments needed to generate technological developments with long lead times have at least three difficult fundamental realities to cope with.

First, the Top Nation (Spain four hundred years ago, France two hundred, Britain one hundred, the United States recently, Japan soon, perhaps China after that) has the easiest job; such a nation can afford to be involved in all important fields, and so does not have to face the politically awkward decisions about what *not* to do. Accordingly, its technology choices are concentrated on the more detailed matter of the picking and efficient prosecution of *project* winners. This is by no means easy, but it is an area where lobbying from those rejected is a little more manageable. Other, smaller economies have to contract out of some important topics because they cannot afford everything, and thus have to begin with criticism from the lobbyists for the items that lose out. This is true whether the rejection is explicit, courageous and rational, or whether it is left to the shorter-term working of the market process.

Secondly, there are two kinds of forecast necessary for these decisions, whether or not they are prepared explicitly. On the one hand, one needs to predict the general climate of social relevance and acceptance for possible new technologies. On the other hand, one has to foresee the relative positions and comparative advantages of different competitors, whether nations or sub-national institutions such as firms or research teams. For the first, one is predicting an environment over which one has little control; for the second, one is in the business of partly self-fulfilling prophecy. A team having cohesion and enthusiasm can, with a less attractive technology, sometimes prevail over another with better propositions but which is bedevilled by internal rivalries and inefficiencies. (This point is forcefully made by Irvine and Martin on p. 144 of their book, where they point out that self-fulfilling prophecy is an important component of Japanese success.) So the two parts of policymaking — trying to predict the weather, and deciding between umbrellas, parasols and other devices — must be to some extent separate.

Thirdly, in retrospect no forecast can be seen to have been absolutely right; even though one protagonist predicts so much better than everyone else that his nation or firm gains great comparative advantage, he will have been wrong in many conspicuous respects and practical success will usually be accompanied by unjust criticism of (often irrelevant) error. This is why the ambitious get into politics and stay out of explicit forecasting if they possibly can. "Act promptly, but never give your reasons; your actions may well be right, but your reasons will usually be wrong." "Treason doth never prosper; what's the reason? Why, if it prosper, none dare call it treason."

Against this background, it is not surprising that books on the conduct and success of forecasting in science and technology make melancholy reading, especially in those countries that once were Top Nation and are now no longer. Of the two books reviewed here, *Foresight in Science* (which should really have been called *Foresight in Technology*) is concerned with national technology policies and decision-making at the macro-level, and only incidentally covers the science that underpins them, while Dr Rotherham's book, *Research and Innovation*, is a record of the Wolfson Technological Projects Scheme which, between 1968 and 1981, allocated a total of £17 million to 186 projects — an average of just under £100,000 each. But, because such micro-decisions determine whether the bright people in universities languish unfunded, devote themselves entirely to intellectual curiosity, or seek to understand and link their work with social needs, the importance of the Wolfson scheme is greater than the size of the sums might suggest.

Many funds finance projects and study; the Wolfson initiative, master-minded by Lord Zuckerman, sought to break new ground by using money to launch teams that could thereafter earn their own living by trading in the technology that they generated. Grants were usually to be once-and-for-all, so that minds were to be concentrated by the prospect of being hanged. Thus, the assessors of the many applications were going much further than the normal process of peer judgement on scientific merit, and were attempting to forecast business success. By comparison with the rest of the academic funding system in Britain, the Wolfson scheme has had, *pro rata*, a good deal of impact on the economy at large. It must, however, be recognized that the Wolfson teams usually drew heavily on the results of work funded by the British Research Councils or University Grants Committee. Thus, Wolfson has sought to set up a series of do-it-yourself "Micro Research Development Corporations" alongside the National Research Development Corporation. The original objective was that each team should survive after the end of the grant by selling its efforts and wares in the market, and to generate capability for innovations rather than for measurement and more orthodox consultancy services. However, this objective has been broadened, for Wolfson has now endorsed as "success" two other kinds of team self-sufficiency.

One is that of those groups which, though not yet trading successfully in the market, have enhanced their thrust and esteem enough to win support from the Research Councils at the end of the Wolfson grant; the other is the achievement of the groups which have met quite new needs in measurement and technical consultancy. This point is illustrated by Dr Rotherham's thumbnail sketches of each of the projects funded, as well as his overall critique, which show that the total success rate of over 50% includes about 10–20% as "innovation contribution", about 20% as "contract measurement and consultancy" and about 20% funded after Wolfson from the public purse. These are impressive results, and we really need a more precise assessment along these lines. Dr Rotherham does not attempt it, and indeed is reflective and descriptive rather than judgemental, possibly on the reasonable ground that comparisons are divisive and that academic dog should try to avoid eating academic dog. His book is nonetheless a most useful quarry for information about the Wolfson units and ideas about adventurous funding methods.

Another comparison can be made between the numbers of awards to different institutions and of resultant commercial successes. Four universities were awarded around £1 million or more: Imperial College, Manchester, Southampton and Birmingham. Of these, Southampton has the biggest yield of market trading, but mainly in the assessment, advice and measurement business. It is, however, rather early to draw firm conclusions about improved methods for project choice. Perhaps it is not unexpected that the pattern of Wolfson esteem for different universities, as measured by grant levels, is very different from that of the University Grants Committee as shown by the pattern of the 1981 cuts in their funding.

Foresight in Science, which was written under contract to the UK Advisory Committee for Applied Research and Development (ACARD), is concerned almost entirely with the machinery used for technical forecasting in different countries, and the notice taken of such forecasts and agencies by the political masters. There is good

justification for the lack of attention the authors give to case detail in that there is fair unanimity in different countries' choice of the technological areas likely to be of most importance during the next few decades. Consequently, most interest lies in the extent to which the forecasts are used, taking national capabilities and disabilities into account in determining policy (if any) in this area.

On this basis, France and Germany emerge as confused and unimpressive; the United States pluralistic and somewhat wasteful; Japan mysterious, purposeful and effective; and the private sector everywhere assiduous but rather blinkered. The snag is that formal forecasting is always supplemented by unofficial, less transparent procedures. In France, for example, the polytechniciens do not seem to pay much attention to the machinery for science funding, and the key decisions — for example those concerning the reconstruction of the auto industry after the collapse of Citroen, the building of a common engine plant, the methods for ensuring the use of French computer equipment, and the early entry of France into microelectronic telephone switching through the system E10 — are not closely linked with the academic scientists and their work. The polytechniciens carefully watched the Colloque Nationale, and the grand Gallic gestures of M. Chevènement. They were then able to cash in on any beneficial trends when they were brought back to clear up the chaos.

In Germany, it seems to be the engineers rather than the scientists whose priorities have most effect on business decisions and are most responsive to them. Again, there are informal alliances which play a large part in German industrial technology but which are not part of official machinery. In the United States, public purchasing of prototypes and funding of private sector research through NASA and the Department of Defense moves very large sums of money in such a way as to lengthen the forward-looking of American firms. However, the policies of President Reagan, especially those currently designed to cut the budget deficit, are eroding energy and other technologies with long lead times. In all three countries, as well as Britain, strong internal rivalries and conflicts have diminished the extent to which choices, once made, are pursued loyally and single-mindedly. France, Germany, the United States and Britain have often failed to enter the recommended and important area of self-fulfilling prophecy.

It is in Japan that formal and informal processes operate hand-in-hand, thereby ensuring that decisions get the maximum chance to work; for example, the development of ceramic gas sensor systems has been helped by a law compelling every house to have a gas leak detector. Japanese weakness in primary, counter-cultural innovation is offset by the prompt identification of successful new ideas from other countries, and early inward licensing while it is cheap. Japanese technologists have surveyed the world, and brought foreign teams to Japan, to encourage competitiveness in their next round of computer and control-engineering development. Perhaps more important still, because of the large proportion of the gross national product that is invested, the Japanese banks are able to insist on long-term technical planning as a condition for loans. Although a very great deal happens that is not visible on the surface, the unity of purpose makes the visible moves a better guide to events and prospects than is the case elsewhere.

What will not be seen for a long time is whether the elaborate, dedicated and expensive Japanese effort being devoted to the science base will bear fruit, or whether social dedication to teamwork and co-operation will inhibit the idiosyncratic innovator. Real originality — the eccentric thinking of the counter-cultural heretic — is not understood or tolerated by a disciplined social machine such as that in Japan today.

Irvine and Martin's book is a valuable source of information on models and systems used in various countries, and how they are meant to work. Confusion, however, arises from their intermingling of attempts to generate policy for technology on the one hand, and science on the other. It is easier to identify technological priorities, and to plan accordingly, than to plan science. Irvine and Martin say clearly that market-pull and ideas-push are needed in collaboration, but the technology does not usually arise sequentially from science (despite the understandable belief to the contrary in the science community); usually, it uses science as a source of strength and reinforcement. Some science planning can be done in response to technological opportunity and need, but this must not exclude curiosity-orientated work, which formerly was cheap enough to be based on peer judgement of the promise of brilliant individuals. Unfortunately, the growing expense of some curiosity-based science is now creating a very awkward need to make choices on the basis of such difficult concepts as "number of major surprises per megabuck". This deeply passionate process must be kept separate from technological planning, or muddle ensues. □

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● Longman has recently published a collection of essays examining the implications for scientific endeavour of the increasing links between academic research and industry. The book is entitled *Science as a Commodity: Threats to the Open Community of Scholars.* The editors are Michael Gibbons and Bjorn Wittrock, price is £9.95 in paperback.

Mathematics and membranes

W.D. Stein

The Physical Chemistry of Membranes.
By M.E. Starzak.
Academic: 1984. Pp.334. $47, £38.
Equations of Membrane Biophysics.
By N. Lakshminarayanaiah.
Academic: 1984. Pp.426. $69, £55.50.

READERS of *Nature* will be aware of the present excitement in membrane biophysics. Our ability, by the patch-clamp techniques of Neher and Sakmann, to measure the electrical properties of single ionic channels in membranes means that the molecular events in channel opening and closing, and in ionic movement through channels, can be monitored. More and more ionic channels are being isolated biochemically, the genes coding for the channel proteins cloned and sequenced, and the channel proteins themselves investigated by physical techniques. Structural models of such channels have been proposed, and the theoretical investigation of ion movement through channels is at a stage where the electrical properties of channels will soon be predictable from them. The study of membrane carriers and pumps lags behind, but pumps have been crystallized in two-dimensional arrays, and the structure of a pump solved, albeit at a low resolution. The dynamics of the membranes themselves are beginning to be understood, the viscosities of regions of cell membranes measured, and the intra-membrane and trans-membrane diffusion of membrane components determined and understood in physicochemical terms.

All this is the meat of university courses on membrane biophysics. I know, however, from personal experience, how difficult it is to convey the sense of new excitement while still providing the firm basis of physical chemistry that students need for a critical appreciation of the modern approach. The subject is of its nature heavily mathematical and quantitative. What, then, is one to do with this mathematics? Write it all out on the blackboard — or merely refer the student to the original (often difficult) paper? Both of the books under review try to solve this problem by providing a text which includes many of the relevant equations. Unfortunately, neither book is really satisfactory.

Starzak's book suffers in part from its misleading title. One would expect that *The Physical Chemistry of Membranes* would treat the membrane's physical chemistry — the forces that mould and stabilize it, its viscosity, the diffusion within the membrane of phospholipids and proteins. These topics are not in fact covered, and the emphasis falls on the surface chemistry

of the membrane and on ion movement across it. The problem, it seems, is that Starzak has felt obliged to deal with a number of general questions of solution chemistry, elementary and irreversible thermodynamics, chemical and enzyme kinetics — even electronics! — on all of which topics excellent books are available at both elementary and advanced levels. Even the relevant topics that he does include, such as ion movements across excitable membranes, are well discussed, at the same level, in other texts. A further defect is the exceptionally poor bibliography. No attempt is made to refer the student to available texts on the subjects covered, and of the 30-odd papers quoted nearly half are citations (sometimes incorrect) of sources from which diagrams have been taken.

Lakshminarayanaiah's book is a more substantial effort. It is comprehensive in scope, covering most of what one would expect to find on ion transport across membranes, with treatment (more elementary) also of non-electrolyte flows. It is hard going in parts, especially where the meaning of a symbol alters over a few pages. I found the treatment of surface potentials quite opaque as these changed names (from distribution to outer voltage potentials, from Lange to contact or from inner to Galvani) and interchanged symbols (from Ψ to Φ to V). The bibliography again is unsatisfactory, being perfunctory and even irrelevant in the earlier chapters. But the main drawback of the book is its style. There are difficulties with the language itself — such as the absence of the definite article — and obscurities in the text from which it is inordinately difficult to wrestle meaning.

There was certainly a place in the literature for both of these books. However, in order successfully to fill this place they should, perhaps, have been more keenly criticized while still in manuscript. □

W.D. Stein is Professor of Biophysics at the Hebrew University, Jerusalem.

Vision interrupted

John Mollon

Visual Masking: An Integrative Approach.
By Bruno G. Breitmeyer.
Clarendon: 1984. Pp.454. £25, $34.95.

MAN's resolution in time is poor. If we are to analyse two successive stimuli separately, or if we are reliably to report their order, then the interval between them must exceed 50-100 milliseconds. Our modern machines have a much better resolution; and at least one specialized subsystem in the human brain — the subsystem that discriminates the directions of sounds — enjoys a temporal resolution that is almost four orders finer than that of the system as a whole.

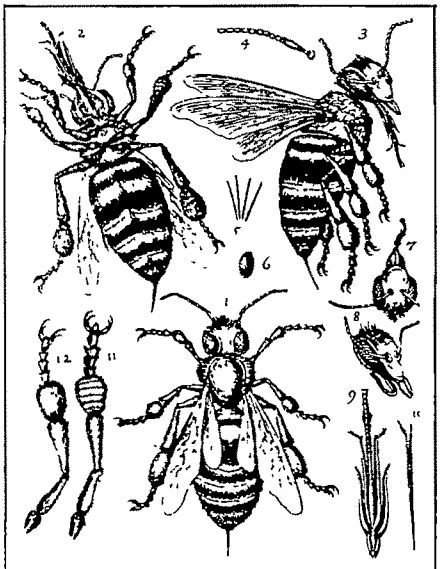
The coarseness of our temporal resolution is well illustrated by "backward visual masking". If a target letter is presented for only one millisecond, it will be easily identified by a human observer, provided it is of sufficient contrast; but if it is followed within, say, 50 milliseconds by a spatially superimposed montage of letter fragments, then it will be masked. Especially curious is the form of masking called "metacontrast": if a bright square patch is presented briefly and is followed within 50 milliseconds by two flanking patches that are of the same luminance as the first patch, then our perception of the first patch is almost completely suppressed.

Such phenomena promise a means to analyse the early processes of visual perception; and therefore masking has been experimentally studied for over a century. There has not previously been a book on the subject, and so it is valuable to have Dr Breitmeyer's comprehensive survey.

Among the topics he covers are persistence of vision, masking by flash and by pattern, metacontrast, the effects of stimulation of the peripheral visual field, and the integration of information from successive fixations. One of Breitmeyer's strengths is his excellent grasp of the classical German literature on masking. He does, however, neglect the French literature, and particularly wanting is discussion of the papers of Jeanine Blanc-Garin. Nor is there any reference to Baylor and Hodgkin's work on vertebrate photoreceptors, a weird omission in a book concerned with the temporal aspects of vision.

Breitmeyer's skill lies in giving accurate short accounts of experiments and theories. However, he could have been more critical of many of the studies included. For example, he devotes several pages to an analysis by Michaels and Turvey that purports to show that the first and second of two stimuli contribute equally to the formation of a "cortical icon". The analysis depends on subtracting the masking curve for the non-dominant eye from that for the dominant eye; in each case, the ordinate is the percentage of trials on which the target is correctly identified and the abscissa is the temporal relationship of target and mask. The fallacy here is the common one of taking behavioural performance as a direct measure of some internal process or state. Breitmeyer unjustifiably assumes that the performance difference between, say, 100% and 60% correct corresponds to the same degree of internal integration as does the difference between 40% and 0%. (If this type of error had a recognized name, it might occur less often. "Fallacy of reification" would capture the essence of it.)

But worst of all, Breitmeyer is silent about an experimental scandal that has marred this field of research. Between 1965 and 1980, many experimenters (including Breitmeyer) uncritically used commercial tachistoscopes that had physical time constants much longer than manufacturers claimed. What happened was that the manufacturers — and those few experimenters who did bother to calibrate their tachistoscope lamps — used photocells sensitive to the fast, short-wave component of the lamp output whereas the eye was maximally sensitive to the slow, long-wave, lamp phosphor. The properties of commercial tachistoscopes were documented in 1978 (*Q. J. exp. Psychol.* **30**, 555) and it would have been good to see Breitmeyer assess the problem as he reviewed the literature. Since 1978, tachistocopes have been largely replaced by computer-driven displays. Astonishingly, however, the persistence of the long-wave component of display phosphors has led to a fresh generation of experimental artefacts. Thus Chapter 10 of the book places great weight on experiments on integration across saccades, but these results had been shown to be artefactual before the date of Breitmeyer's preface (see, for example, *Science* **222**, 188; 1983).



Nature magnified — enlarged views of the honey-bee, the earliest known studies made with the aid of a microscope, from Francesco Stelluti's Descrizzione dell'Ape *(1625). The illustration is reproduced from* Single Lens, *by Brian J. Ford, which will be reviewed in* Nature's *Spring Books issue on 25 April.*

Breitmeyer finally gives himself away in his long "Epilogue", a rambling, overblown piece of bull in which Aristotle, Galileo, Descartes, La Mettrie, Kant, Goethe, Santayana, Bergson, Monod, Tolman, Lorenz, Piaget, Polyani, Popper, Dawkins, Breitmeyer and others, jostle promiscuously. It is troubling that the publisher did not have the editorial firmness to delete this embarrassing 20-page essay. □

John Mollon is a Lecturer in Experimental Psychology at the University of Cambridge.

Good vibrations

Colin Gough

The Physics of the Violin.
By Lothar Cremer.
MIT Press:1985. Pp.450. $35, £41.95.

FOR over 150 years scientists have been investigating the physics of the violin. Some have been motivated by the hope of discovering the "Stradivarius Secret" — the scientific explanation of the highly-valued tone quality of many instruments of the famous Cremonese violin makers of the seventeenth and eighteenth centuries. For others, such as the author of this book, the attraction has been to understand the acoustical and vibrational physics that ancient luthiers empirically incorporated into their instrument designs, culminating in the "modern" violin around 1550.

In the nineteenth century, musical acoustics was at the forefront of science. People of the stature of Helmholtz and Rayleigh, often through their interest in acoustics, were laying the wave-mechanical foundation of modern physics. And in the early part of this century, Raman, later to win the Nobel prize for his work on optical spectroscopy, published his first scientific papers — on the physics of the violin. But thereafter, with the advent of the exciting new fields of quantum science, musical acoustics became something of a backwater.

In recent years, however, the subject has experienced something of a revival, arising partly from the recognition of a number of interesting problems of "classical" physics still to be solved, and partly because modern experimental and computational techniques now allow a much more realistic modelling of the science involved. Prominent in such activities have been Professor Cremer and his co-workers at the Institute of Acoustics in Berlin. Over the past 25 years, this group has published a succession of important papers on musical acoustics. Now Professor Cremer has summarized that work and has brought together material that was hitherto widely dispersed in the literature. Although no attempt has been made to provide a fully comprehensive coverage of current research, generous space is devoted to closely related work of other authors.

Cremer treats the violin simply as a mechanical structure that is forced into vibration by the bowed motion of the string. Almost half of the 450 pages are devoted to the bowed string problem, reflecting the author's main research interest. The transformation of vibrational energy by the supporting bridge, now widely recognized to be a major factor in determining the tone of an instrument, is discussed in detail, as are the vibrational modes of the instrument as a whole. This logical approach to the energy chain is concluded with a discussion of sound radiation and the way that this sound is modified by the environment.

Professor Cremer hopes his book will appeal not only to the scientist but also to the violinist or violin-maker wishing to understand the underlying science of their instrument. Unfortunately, few musicians or craftsmen are likely to possess the mathematical background to cope with the relatively sophisticated theoretical description of the complex vibrational characteristics of the violin. On the other hand, the book should certainly convince any scientific reader that musical acoustics, and the acoustics of the violin in particular, is a perfectly respectable and fascinating field of applied science; indeed recent research on the violin has led to a number of new ideas that could usefully be applied to many other vibrating systems.

Cremer makes no attempt to relate physical measurement to the subjective assessment of a violin's quality. As he explains in the preface, although modern science can provide a fairly accurate description of the physics involved in producing sound, we simply do not have the necessary language even to start to try and correlate them. This is a challenge which will probably keep physicists, psychoacousticians, players and instrument-makers busy for at least another 150 years.

Colin Gough is Senior Lecturer in the Department of Physics, University of Birmingham.

Power and penguins

David J. Rose

A Guide to Nuclear Power Technology: A Resource for Decision Making.
By Frank J. Rahn *et al.*
Wiley: 1984. Pp.1,000. £101.30, $106.

NUCLEAR steam supply systems have millions of parts, far more than Boeing 747s and in their way just as high technology. Describing this technology in a single book, albeit a big one, is a daunting task— some might say impossible. But it is the subtitle, *A Resource for Decision Making*, that is the reason for my concern about this book, of which more anon.

What is there here, in 500,000 words of small print plus 900 figures and tables? In 20 chapters, from basic processes, through radiation, the fuel cycle, reactors, materials, operation, safety, health and environment, regulation, economics, proliferation and intermediate stops, the book describes a particular world of nuclear power, often in more detail than we want. It is mainly an American world, of diagrams, photographs and words about all these subjects. Not wholly, of course, because some topics such as hydrogen flammability limits and radioactive toxicities know no national boundary. But the Canadian CANDU reactor gets a mere eight pages, the British Advanced Gas Reactor four pages and the French reactor standardization one 10-word clause on page 799. Comment on the Far East, where many innovations are taking place, seems to be missing completely.

Even the great specificity about equipment and practices in the United States is not very well matched to the stated task of informing decision-makers. The book describes in great depth how things are now, somewhat mechanically: properties of steel, operation of the latches on a control rod drive, patterns of refuelling a light water reactor, an extractor evaporator for removing water from rod waste and a myriad of other things. Most of this, like the mythical book about penguins, gives the decision-maker more detail than he wants. It isn't wrong, but is it useful?

Decision-makers want to know other things. Why did the system work (or not) before? What are the present options? What are the trends? Compared to what? Here are a few examples.

- Why do nuclear power plants cost so much, when the entire nuclear steam supply system plus the turbine-generator costs only about 25% of the total? And why such large disparities from place to place? The chapter on economics lists increases in material costs, effect of lead time and so on, but still leaves a fuzzy impression.
- What about the prospect of substantially improved light water reactors for the near term? The work proceeds now, principally in Japan, aiming for higher capacity factor, less radiation exposure to workers, easier maintenance, more compatible materials and so forth. But I find nothing of this, only some four pages on such topics as the classic spectral shift idea. If decision-makers in the United States don't watch out, the initiative will pass from them, surely something they should know.
- The potential of smaller reactors is becoming an increasingly lively topic these days. Is there the possibility of overcoming the diseconomy of small size by the economies of serial, factory or modular construction? Such smaller reactors would decrease the financial exposure of electric utility companies. Again, there is nothing on the subject here.

Still, the book has its merits and can be useful, though not principally as a handbook for decision-making, let alone policy decisions or technology assessment. It is descriptive and not overly technical (as its preface says), and fairly well written. Of the twelve groups to whom the authors commend the book, two seem most likely to profit from it: engineers with an interest in but no special training in nuclear technology, and nuclear engineers wishing to get a broader grasp of certain aspects of nuclear technology. □

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nature

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Self-reliance means self-denial

The Indian government seems to be changing its policy on the import of technology. Far from undermining Indian hopes, this may be the best assurance that they will come true.

To what extent should developing countries seek to keep up with the industrialized countries of the world in technology? And how should they attempt to do so? Unhappily, there are many places in the world where the question is strictly academic. For most of Africa south of the Sahara, for example, the notion that indigenous science and technology might contribution to the solution of immediate problems is, for the time being, an impossible dream (which is not to say that it should be forgotten). But there are other states, of which India is the chief example, where governments have for decades sought to drag themselves into the future by devoting resources to research and development. Often, as a consequence, they have run into criticism from those elsewhere who complain that when resources are so scarce, they should be spent instead on more immediate tasks, the relief of poverty perhaps. That argument is mistaken (and patronizing as well), but that does not absolve developing countries from working out sensible policies on research and development.

The need is illustrated by the public row in India during the past month over the new government's policy on semiconductors. At budget-time last month (see *Nature* 4 April, p.496), the Indian government announced several measures to liberalize the import of technological equipment from overseas — computers, for example, and also announced an arrangement with the Hemlock Semiconductor Corporation of the United States under which the overseas company will build and license a polysilicon plant in India. This decision has aroused opposition within India from three sources — those parts of the Indian research establishment that complain that the deal demeans their work, industrial companies that consider the arrangement an unfair challenge which robs them of opportunity and those who argue more generally that dependence on overseas technology is inappropriate for a country such as India. The government's resolution of the argument, announced last week, is a compromise: the arrangement with Hemlock will go ahead, but the Mettur company, which has built a 2-tonne a year pilot plant for manufacturing polysilicon by a process developed at the Indian Institute of Science at Bangalore, is also to be encouraged to build a 25-tonne plant at Baroda. Meanwhile, according to the electronics minister, Mr Shivraj Patil, the indigenous technology will be further encouraged by the placing of research contracts with laboratories such as the National Chemical Laboratory at Puna. By what tests does it make sense to follow both paths, keeping a dog but being prepared to bark oneself, so to speak?

Self-reliance

The answer, convincing enough, is complicated, and turns on the distinction commonly made in India between self-reliance and self-sufficiency. Self-reliance is that state of grace in which a country embodies the technological skill needed to accomplish all known goals, whence the considerable Indian investment in space research. Self-sufficiency is more demanding, requiring not merely the skill but the capacity (technical and financial) to do everything. Since independence in 1947, Indian governments have been wedded to the doctrine of self-reliance, and have also flirted from time to time with that of self-sufficiency (whence, in part, the cumbersome system of import controls now being dismantled).

The doctrine of self-reliance is especially confused in its application. India's need, like that of other developing countries, is for a degree of technical competence that will suffice to meet the unknown demands of the future. One ingredient of any provision to this end must be institutions at which scientific work (teaching as well as research) of the highest quality is carried on. India is reasonably but not lavishly provided in this respect: there is a handful of fundamental research establishments of international standard, but the quality of training and research within the university system is too patchy to ensure that the fullest use is made of talent. More serious questions arise in the application of technology. Since the 1950s, India has maintained its large nuclear energy establishment for two declared reasons — to provide a model of the indigenous application of technology in a daunting field, and to maintain independent control of an industry whose importance ranks high, politically and economically. In the first respect, the policy has worked well: the Indian Atomic Energy Commission is an important source of technical expertise. But India would have had more rapid access to nuclear power if it had been able to sign contracts for building power stations with overseas suppliers and to put up with controls on the use made of plutonium. This is the lesson that Mr Rajiv Gandhi now seems to have applied in the smaller matter of the semiconductor industry.

Prudence

In prudence, he should now go a step further. A huge half-developed half-developing country such as India has two needs of technology — the chance to manufacture products that compete on world markets, thus earning the foreign exchange needed to pay for imports, and the promise that, in the future, there will be even more sophisticated things to make and sell, domestically and overseas. Traditionally, Indian governments have sacrificed the first need to the second. The notion that India might successfully build an industrial base by licensing technology from overseas, and only afterwards embellish that with its own distinctive contributions, has often given great offence. Yet this is how many other modern states have prospered. In India, a further benefit of a policy typified by the Hemlock deal would probably be that Indian technology would be improved in those respects in which it is at present weakest, the management of projects. Mr Gandhi's government should build on the precedent it has now established, looking for opportunities to benefit from technology now, not later Far from being a threat to what may lie distantly ahead, a policy of judicious exploitation of other people's technology is a necessary foundation for the launching of more ambitious enterprises in the future.

The bearing of the Indian problem on the condition of other developing countries is remote, but not entirely negligible. While the world wrings its hands over the famines sweeping much of Africa, it tends to be forgotten that the agricultural deficits that afflict them have been outlawed in India by the intelligent application of technology, that of agriculture. Experience not just in India but in the United States has shown that the improvement of food production requires not merely technical innovation (new seeds especially) but means by which farmers, often peasant farmers, can be helped to use them efficiently. Yet during the past twenty years, this combination of research and extension service has been eroded in many of the countries of West Africa

where it was once productive. Many other developing countries have never benefited in this way. The moral for them is that priority should be given to the development of agricultural research. The other leaf that should be taken from the Indian book is the demonstration of competence in the pursuit of science, if only so that too much talent is not lost too soon. □

Powell bites dust

The British government needs to think hard now about surrogacy.

THE British government has done the decent (and courageous) thing by deciding that it will not lift a finger to save Mr Enoch Powell's bill on *in vitro* fertilization from being lost in the procedural hazards of the House of Commons (see p. 573). Common sense requires that there should be an opportunity to consider carefully how research in this field should be regulated. That regulation of some kind is necessary is agreed by everybody, even those who work in laboratories, but there are serious doubts whether the 14-day limit suggested by the Warnock committee is either appropriate or workable. It is to be hoped that the government will now consider seriously whether regulation by public ethical committee would be preferable, not least as a way of meeting the public concern that research with living human embryos should be responsibly designed and soberly directed. That course will not comfort those (Mr Powell included) who hold that the whole idea is an abomination. That is the sense in which the government has shown courage — and is the reason why prudence again requires that it should press ahead with its own legislation as quickly as it can.

To take the edge off the complaints there will be when the Powell bill is defeated next month, the government plans that there will be an outright ban on what is called commercial surrogacy, the practice by which healthy women offer the service of gestation to others for a fee. Warnock would have banned all surrogacy, but an important minority opinion on the committee held that some allowance should be made for exceptional circumstances. The plain truth, now, is that nobody knows how common may be the practice in countries such as Britain, and there is only guesswork to suggest what may be the circumstances (apart from the infertility of the intended mother). Yet reports abound of women providing this service for infertile sisters, while there is no way of knowing what small proportion of illegitimate births followed by adoption are essentially surrogacy arrangements.

That is one reason why the outright ban on "commercial" surrogacy now proposed deserves more careful consideration. In the spirit of the present time, which rightly allows an adopted child to learn something about its natural parents at majority, frankness about genetic origins is in fashion (and could, sometimes, be medically important in tracking genetic defects). Should these principles not also apply to children born of surrogacy in the forms now accepted? Will such practices, tacitly condoned at present, become crimes if the surrogate mother is rewarded in kind, not cash? And what is to happen if the small but unavoidable risks of pregnancy to the mother should become realities? Do her dependants go neglected? These questions may be hypothetical, but they are also important.

The British government has started from the other end of the problem — the publicity and the attendant fuss following a surrogate birth last year in which a commercial agency had been involved. Would the fuss have been as great if the agency had been a registered charity, organized perhaps as if it were an adoption agency? Almost certainly not. This is but one reason why the British government should be prepared to allow for exceptions to the rule it is now planning to introduce. The guiding principles should be that surrogacy should not be allowed to make anybody's fortune but that women who volunteer to provide the service should be safeguarded and repaid for their time and trouble, preferably in a manner open to public scrutiny. Is that too much to ask? □

International manners

The US administration should not offend its friends overseas by tactlessness.

NEXT month's meeting of the heads of government of the major industrialized nations, to be held at Bonn in West Germany, has all the makings of a rough-house. On this occasion, the hard core of the agenda, economics, will be the chief reason why the United States will find itself pinned uncomfortably in a corner. But US policy on strategic arms is likely also to sour what people have to say to President Ronald Reagan. Continuing doubt over US intentions over its star wars plans is one bone of contention. Italian President Bettino Craxi was right to complain last week that the US administration owed Mr Mikhail Gorbachev a less ungracious reply to the Soviet offer to suspend the deployment of SS20 missiles aimed at Europe than the jaundiced comment that it was a mere propaganda ploy.

The substance of these issues has been much debated, here and elsewhere, but the manner in which the United States now conducts its international business is becoming an obstacle to its attainment of its objectives. Even those who agree with what the administration is trying to achieve must be dismayed. Mr Gorbachev, after all, declared that the Soviet Union would unilaterally halt the deployment of SS20 missiles within range of Western Europe until November, and would continue a moratorium thereafter if mutual agreement was reached by then. The proposal is full of snags, while the plan to make it public at this stage is a breach of the agreement between the superpowers to restrict their negotiations on strategic arms to the closed sessions at Geneva. But even a thoroughly sceptical administration could have contented itself with something like "High time!" or "There'll be time to talk about that at Geneva". Last week's needlessly hard lines will have made many third parties ask who are the bad guys now.

Much the same is true of the US administration's public statements on the international money problem. In the past few days, US cabinet officers have too quickly taken up President Reagan's cry last month that there would be no imbalance in the world economy if only European states were as productive as the United States, which is at best a half-truth and which may even be more false than true. (If Europe were as productive as it might be, the United States would not be able to fund its present deficit by borrowing from abroad except by pushing interest rates still higher.) The irony in this is that the United States seems not to have been restrained in its attempts to correct its trade balance with the most productive of its international competitors, Japan. But the proposal now being canvassed by officials in Washington that would-be US exporters should be represented on the regulatory committees that will deregulate the supply of equipment to the Japanese telephone system will seem especially demeaning in Japan, where it will be recognized that the proposal would be constitutionally impossible in many places, and in Europe, where it will be asked why only US exporters should enjoy this privilege.

Complaints such as these that the United States too often behaves as if its own interests are paramount are not new. Most people also acknowledge that the United States has a special role in world affairs, militarily and economically. The sympathetic among them also appreciate the difficulty of a government such as that of the United States, with huge domestic preoccupations and watched over by a necessarily parochial Congress, in finding forms of words to explain itself that do not raise hackles elsewhere. Yet the administration's performance seems to be getting worse.

Part of the trouble may be the number of new faces, or of old faces in new jobs, that have made their appearance at the White House and in US government agencies since the beginning of the year. That is the charitable explanation, in which case Bonn may be a stimulus for changing tack and for learning that international relations are as easily soured as personal relations by tactlessness. Let us hope the explanation is not more sinister. □

Research secrecy

Pentagon plays havoc at optical conference

Washington

THE US Department of Defense (DoD) caused severe disruption at a technical symposium of the Society of Photo-Optical Instrumentation Engineers (SPIE) in Virginia last week when it withdrew at a few days' notice some 50 papers previously scheduled for presentation. The titles of the papers and some of the abstracts had been cleared for publication by DoD, but many of the texts were later found to contain either classified information or information subject to export regulations.

Eventually, most of the papers were presented (after some censorship) in a new type of "export-controlled" session, attendance at which required US nationals to certify that they would keep technical information confidential and which required foreign nationals to be sponsored by their embassies. Twelve of the 50 papers were declared classified and were not presented.

DoD last year declared that it would rely only on classification to restrict publication of fundamental research carried out on university campuses. As universities do not generally carry out classified research on campus, this seemed like a guarantee not to interfere with academic work. But the papers restricted at the SPIE conference were based on research by private contractors receiving DoD funds in category 6.3, which covers research that is potentially militarily sensitive and classifiable. Research in category 6.3 is not generally considered to be "fundamental", but none of that affected at the SPIE conference had actually been classified. Private research contractors, in contrast to universities, do accept contract clauses requiring researchers to submit papers to DoD for prepublication scrutiny — effectively a licence for DoD to prevent dissemination of the information should it so choose.

DoD says that all of the 50 affected papers would have had to be suppressed totally, by invoking prepublication review clauses, but for a new authority in the 1984 Defense Authorisation Act, under which DoD could allow most of the papers to be given, but on condition that those present would agree to the confidentiality requirements of export-controlled sessions.

Conference organizers say that no foreign national wanting to attend one of the two export-controlled sessions was denied access, but a number of them at the conference decided, for various reasons, not to seek admission to the controlled sessions, which dealt with synthetic aperture optics and wavefront sensors. Both areas have applications to astronomy as well as to weapons design: adaptive optics may be used to "untwinkle" stars in large telescopes, while synthetic aperture interferometry may allow many optical telescopes to be combined.

In the 1984 Defense Authorisation Act, DoD was given new authority to deny public access to unclassified technical data in its control that are judged to contain critical technology with military or space applications and which cannot be exported without restriction under Export Administrations Regulations. Previously, the department had no authority to withhold such technical information when faced with a request under the Freedom of Information Act.

It is now DoD policy to oppose the open distribution at conferences of unclassified technical data subject to export control. But DoD is also authorized to make such data available to those who are prepared to guarantee its confidentiality, which mechanism DoD brought into play for the first time at the SPIE conference. The exemption was granted by Congress in response to the concern of small defence contractors, which feared that they would otherwise be denied data necessary for them to bid competitively for DoD contracts.

The American Civil Liberties Union and the American Association for the Advancement of Science expressed concern last week that DoD had used a legal authority applying to DoD-controlled data to restrict public access to research material still the property of the contractors. DoD's explanation is that it still relies on contractual restraints to prevent open publication, and that only the exemption of those guaranteeing confidentiality was derived from the 1984 Authorisation Act. But, paradoxically, the exemption mechanism that DoD brought into play last week could in practice allow DoD to impose tighter control through contract mechanisms. Many allied countries fear that the United States is tempted to restrict foreign access to cutting-edge technology because of its commercial rather than its military importance.

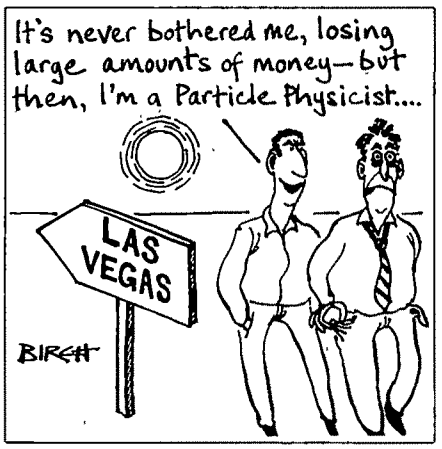
Much of the very visible annoyance at last week's conference was due to the lateness of DoD's intervention. Sessions were being rearranged from one day to the next. But DoD says this was a practice run at making some export-controlled data available in unclassified but restricted sessions, and says that the lateness was due to the failure of another scheme which had been proposed — to have two entirely classified sessions running in parallel with the regular conference. Only one classified session was in fact held. **Tim Beardsley**

Accelerator scrutiny in prospect

Washington

THE US House of Representatives Science and Technology Committee has warned that it intends to treat particle physicists sceptically when considering construction of the proposed Superconducting Super Collider (SSC).

In approving the administration's request of $20 million for continued research and development on the accelerator project, much less than the $60 million that the Department of Energy (DoE) had asked for, the committee said that it doubted whether DoE's goal of starting construction by 1988 could be met.



Still smarting over its experience with ISABELLE, Brookhaven National Laboratory's $200 million fiasco, the committee said it would not even consider a proposal for SSC until DoE could prove that the superconducting magnets will function as planned for the advertised price. ISABELLE, delayed for years, was abandoned in 1983 after unexpected problems in design of the superconducting magnets. Although the problems were eventually ironed out, events in physics had in the meanwhile made ISABELLE obsolete, but not before a 2¼-mile-long tunnel, control room, experimental areas and magnet production facilities had been built.

Optimistic projections put the cost of the SSC magnets at 15 per cent less per unit of length than those now operating at Fermilab; at $5,000 per metre and assuming an 80-km ring, that works out at $400 million for the magnets alone. The current estimate for the total cost of SSC is still $3,000 million, not including detectors, research and development and allowance for inflation.

Leon Lederman, director of Fermilab, said that previous proposals had been approved by congressional committees almost "by return mail". "We're bearing the cross of ISABELLE", he said.

Stephen Budiansky

French geoscience

New umbrella wins friends

FRENCH oceanographers, geophysicists, geologists, astronomers and space scientists have clubbed together to form a new organization, INSU, the Institut National des Sciences de l'Univers, which will manage big research programmes under the financial guidance of the principal French research council for basic science, CNRS (Centre National de la Recherche Scientifique). INSU should give academic oceanographers, at least, more weight than hitherto in French science politics.

The institute has been created only after a fight from geophysicists. INSU is based on its predecessor INAG (l'Institut National d'Astronomie et de Géophysique), where the small but effective French community of geophysicists played a strong role. INSU, to their chagrin, includes the more traditional field geologists who outnumber the geophysicists more than ten to one. Some geophysicists, who see themselves as the vanguard of French geoscience, have strongly resisted the move. The struggle continues in the universities and in the laboratories of CNRS, where geophysicists argue that geologists should relinquish more of their posts to the "new geology".

Within INSU (which will be directed by meteorologist André Berroir of the Ecole Polytechnique), realism prevails, and the talk is of new big programmes that will unite the warring tribes along the lines of the recent survey of Tibetan geology and geodynamics headed by Claude Allègre of the Institut de la Physique du Globe. Possible programmes for 1986 include a study of Caribbean tectonics and work in Latin America on the Cordillera.

Geologists themselves will also benefit from the creation in INSU of a working group on medium-scale computers for the earth sciences. Undersupplied with computers, as are many French scientists, geologists expect to need powerful minicomputers to process image data from SPOT-1, a new-generation French Earth observation satellite to be launched later this year. Both science minister Hubert Curien and Berroir, the INSU director, are in favour, even if that means using foreign machines. (Berroir masterminded the first French purchase of a non-military Cray-1.)

Oceanographers feel that the institute will strengthen academic science. In France, the field has been long dominated by the technological interests of CNEXO (recently transformed into IFREMER, the Institut Français pour l'Exploitation de la Mer, by the addition of fisheries research.) INSU should provide oceanographers with some political power, authority and even money with which to strengthen the pursuit of basic science in the face of IFREMER's largely applied bias. Hitherto, they have had to rely on a small understaffed interdisciplinary programme of the CNRS, the "PIROCEAN" programme.

PIROCEAN, with its FF 10 million (£1 million) budget, will now be absorbed into INSU, where it will add to the FF 35 million (£3.5 million) brought by INAG and undoubtedly gain greater influence than it had when directly under the CNRS umbrella.

The new merger should also comfort CNEXO oceanographers whose 450 technical research staff were in danger of being swamped by the 600-700 scientists of the fisheries research organization in the earlier IFREMER merger. (That union also squeezed the research budget of both organizations, as salaries of the old fisheries organization were raised to match CNEXO's without a corresponding increase in total funds.)

Astronomers, meanwhile, who looked to INAG to support observatories and new telescopes, remain blissfully unconcerned. (French membership of the European Southern Observatory is paid for by the foreign ministry.) Plans are afoot for THEMIS, a high spatial resolution polarizing solar telescope for Tenerife (in collaboration with the British, Sweden, Denmark, Spain and West Germany) and for the completion of the millimetre-wave interferometer IRAM in the south of France (due to operate as an interferometer some time in 1987). But this year will be the first for which the astronomers (and geophysicists) have had to fight for large programme funds with geologists and oceanographers. The financial effects of the INSU merger will not be clear until the budget for 1986, due this autumn.

Robert Walgate

UK biotechnology

Public lab. makes private deal

ONE of the most unusual arrangements yet for turning public investment in research into industrial channels, Porton International, announced last week an arrangement with the British government that gives it the right of first refusal on biotechnology innovations at the Centre for Applied Microbiology and Research (CAMR), the laboratory at Porton Down in Wiltshire where British research in biological warfare was once carried out, but which was transferred to the Public Health Laboratory Service seven years ago. The same stretch of Wiltshire down remains the site of the Chemical Defence Research Establishment, operated by the Ministry of Defence.

The new arrangement is the outcome of several years of negotiation, most recently a process in which other major British companies had been asked to express an interest. Porton International already has agreements with CAMR for the exploitation of two of its proprietary developments, asparaginase (used in cancer chemotherapy) and a vaccine for herpes simplex virus (based on developments at the University of Birmingham as well as at CAMR).

In a joint statement, the company and the Public Health Laboratory Services Board said that the new arrangement will run for thirteen years from 1 April, and that the arrangement might be continued thereafter if neither party saw fit to pull out. A spokesman for Porton International said that developments not taken up by the company would remain the property of CAMR, and might be offered to other companies. But he suggested that such happenings would be few and far between.

The management of the laboratory will remain the independent responsibility of the director, Dr Peter Sutton, the Public Health Laboratory Service and ultimately the Department of Health and Social Services. But the existence of the new agreement is likely to divert the scientific interests of many members of the laboratory into directions that may be profitably exploited.

The agreement covers not merely biological materials produced by innovative methods but also new processes that may emerge (as for the manufacturer of phenylalanine and the intra-conversion of steroids). Apart from the materials already being brought to the market, there are plans for the production of frozen cell pastes (for the derivation of restriction enzymes), the production of enzymes (such as carboxypeptidases), vaccines other than herpes simplex (such as against pertussis virus) and cloned human hormones (such as human thyroid and follicle-stimulating hormones).

Under arrangements established before 1 April, Porton International is already financing the work of several members of CAMR. Nobody is as yet prepared to guess how large a proportion of the total cost of running the laboratory will derive from royalties provided by the company on sales of proprietary products. But a spokesman for the company guessed that more than half the 250 members of staff at CAMR were working on projects with some commercial interest, but that the laboratory's basic research was also essential to the success of the new arrangement.

Porton International Ltd is for the time being a holding company, with a number of subsidiaries (such as LH Fermentation) active in fields related to biotechnology. The recent development appears to have been financed by capital contributions from a number of financial institutions, including the pensions funds of several public companies and a number of insurance companies. The chairman of Porton International is Wensley Haydon-Baillie. □

Israeli science

Optimism from new minister

Rehovot

ISRAEL'S new science minister, Mr Gideon Patt, has a great many plans but very little money with which to carry them out. Nevertheless, he remains optimistic.

"As minister of commerce and industry", he declares, "I helped promote applied research. Now, as minister of science, I hope to boost basic research. And just as I initiated a five-year plan for industrial development in my old post, I now intend to initiate such a plan for the development of science and technology".

Patt points out that Israeli investment in science and technology now stands at 2.2 per cent of the country's gross national product (GNP), which is, as he puts it, "a respectable figure", and more than is invested, for example, by the Netherlands. But he hastens to add that the percentage figure is misleading on two counts. First of all, Israel's whole GNP is not large and so the total annual investment in research and development is around US$700 million, less, that is, than is spent on research and development by some individual US companies. Moreover, more than half of the available funds go to military research and development, from which the economy benefits to only a limited extent.

In spite of the problems in regard to applied research, Patt is far more concerned with the decline in funds available for basic research. He notes that the universities, where almost all basic research takes place, once allocated some 50 per cent of the funds for education and the other 50 per cent for research. In the past five years, however, because of massive cuts in government support, they have greatly reduced the money they allocate for research.

"Thanks to superhuman efforts by our scientists", Patt says, "we have not so far fallen behind. However, sheer devotion and ingenuity cannot make up for the lack of funds indefinitely and if something isn't done soon to remedy the situation, Israel will become a scientific backwater, just another little country with enormous problems and few resources with which to solve them."

Patt's solution, one already on the stocks, is to create a special $250 million fund from overseas sources for scientific research in Israel. This will yield up to $30 million a year to support research, a sum that will be supplemented by another $10 million from Israeli government and binational sources.

Why is Patt, a non-scientist, interested in basic research? "I'm not", he says, "I simply realize that without basic research, there will be no innovative applied research, no original products. And if we just base our industry on secondhand research, we will never be able to keep up with our competitors."

The science minister is certain that proper support for research will pay off, generating enough income, through new products, to repay investors in his special fund and to strengthen the economy.

Ultimately Patt would like to see his ministry of science turned into a ministry of science and technology. This would, he believes, facilitate the kind of overall planning that does not exist at present. More specifically, the chief scientists now found in most ministries, where they are charged with helping to find science-based solutions to ministerial problems, would be linked directly to the ministry of science and technology and would be guided by the overall goals that it sets.

In the meantime, Patt has already taken a first step towards greater coordination by creating a regular forum where all the chief scientists meet under his chairmanship at regular intervals. He is now planning to expand the scope of this forum by inviting university vice-presidents for research and development to participate.

Patt has also turned his attention to the shortage of trained engineers for Israeli industry, which already needs 2,000 more than it can find. He supports attempts to bring back some of those now in the United States, though he sees this, at best, as a partial solution. He has therefore joined hands with the army and with the manufacturers' association to establish a special school where practical engineers with two years of post-secondary training could be turned into qualified engineers in two years. But this school may never be created because the universities have tentatively reversed their long-standing opposition to such a scheme and will probably inaugurate a similar programme themselves.

But the new science minister sees real significance in his present position. "Ten years ago", Patt declares, "the ministry of science wasn't very important. Today it is already important. Ten years from now — if the country develops as it should — this ministry will be among the most important in the government." **Nechemia Meyers**

US telescopes

Caltech takes Texan wind

Washington

PLANNERS of new ground-based optical telescopes in the United States have been given pause for thought since the announcement in January from the California Institute of Technology of plans to build a 10-metre segmented-mirror instrument on Mauna Kea in Hawaii. The Caltech telescope, for which $70 million has been pledged by the William M. Keck Foundation, will be the largest optical telescope in the world. Fund-raising for other telescopes is likely to be harder as a result, and some plans may have to be rethought.

The Association of Universities for Research in Astronomy Inc. is doing preliminary design work for the National New Technology Telescope (NNTT), an instrument with four 7.5-metre mirrors that would be built by the National Science Foundation (NSF). But it will be difficult to persuade NSF and the Congress to vote $150–$200 million of federal funds for NNTT unless it offers US astronomers facilities that are clearly superior to those available elsewhere.

As things stand, NNTT would have twice the light-collecting power and 1.5–2 times the angular resolution of the Keck telescope. The question of whether that factor of two is sufficient to justify spending money on NNTT has yet to be decided, according to Laura Bautz, director of the division of astronomical sciences at NSF, who is forming a committee to examine the questions raised. Caltech is preparing a site for a second 10-metre telescope alongside the Keck telescope which, if it were built, might be combined with the Keck telescope for optical interferometry; such a combined facility would have characteristics roughly similar to those of NNTT.

Other telescopes have also been put into the shade by the Keck bequest. The University of Texas has, since 1979, been planning a 7-metre single-mirror telescope at its McDonald Observatory in west Texas, but has so far failed to raise the $50 million needed. The Texas state legislature, which is in the throes of a spending crisis, recently turned down a second request for $5 million of state funds to purchase the primary mirror for the telescope.

The publicity surrounding the Keck bequest will "undoubtedly make it harder" to find a private foundation willing to support construction of the Texas telescope, according to Harlan Smith, director of the McDonald Observatory. The telescope can no longer tempt potential sponsors with the glory of supporting the biggest telescope in the world (although Dr Smith points out it would still be the largest monolithic mirror telescope). Dr Smith argues that the supply of optical telescopes is not comparable with the supply of accelerators for high-energy physics, where there is continual pressure for ever-higher energies; rather, the pressure on optical telescopes is for observing time.

Even the Texans' claim to be building the world's biggest monolithic mirror telescope may shortly be disproved, however. The University of Arizona is expected shortly to announce a partnership with other institutions to build a 7–8 metre monolithic mirror telescope, and the Mount Wilson Observatory in California hopes for a similar instrument. **Tim Beardsley**

IVF

Australian export service

Canberra

THE *in vitro* fertilization (IVF) technology that produced that first freeze-thaw embryo and donor-embryo babies will soon become commercially available to infertile couples in the United States. The vice-chancellor of Monash University in the state of Victoria announced in Melbourne last week that an agreement had been reached, after 18 months of discussion, to form a private company to establish clinics, train US doctors and make available the techniques developed at the Queen Victoria Medical Centre by Professor Carl Wood, Dr Alan Trounson and their colleagues.

The company will be called IVF Australia (IVFA) and its first clinic is expected to be set up in New York. In the absence of more stringent local laws controlling IVF, the services on offer will conform with the guidelines set out in the Infertility (Medical Procedures) Act (1984), which will be proclaimed by the Victorian State Parliament in the current session, having been deferred early last year when state opposition parties successfully blocked its passage.

The bill specifically forbids surrogate embryo transfer and the embryo flushing procedure known as lavage (or *in vivo* fertilization), where fertilization is carried out in the womb of one woman and the resulting embryo flushed out and transferred to the womb of another woman. To achieve bipartisan support, the Victorian Labor government was also obliged to deny IVF treatment to couples in a stable *de facto* relationship, except those already embarked on the Monash programme before the act comes into force. This appears to rule out all US *de facto* couples as well as single women.

With more than 200 babies born at a time when most US clinics are closing down, the Melbourne researchers have brought several procedures to a high rate of success. The services offered will initially involve husband-and-wife gametes only, but will probably be extended to donor procedures as well, and will include embryo freezing and storage.

The Victorian legislation is based principally on the recommendations of the Waller committee, set up to consider the ethical and legal issues raised by *in vitro* fertilization (see *Nature* **311**, 288; 1984). The act bans any form of cloning and any p cedures where human gametes are mi with those of an animal. A couple may minate a (willing) donor, the wom sister for example, but no donor r receive payment beyond normal expen

The Waller committee recommen that research on embryos should ext only over the first 14 days. The Victo government has not yet formulated a reply to the committee's recommendati but the draft legislation provides fo Standing Review and Advisory Commi to be created to consider requests for proval of research with embryos.

Where does the act leave the frozen F embryos (see *Nature* **309**, 738; 1984), wh biological parents perished in an air cr leaving no instructions for their ultim disposition? According to the dr Victorian legislation as it now stands, embryos would be made available to ot couples on the IVF programme who wc be told that the embryos are unlikely grow to maturity because the methods u to freeze them were not as well develo as those now available. The prospec social parents would be supplied with n identifying information about the bi gical parents (the Rios) and if a per grown from one of the Rios embryos w to seek this information from the Victo State Health Commission, it would alsc non-identifying, subject to the discretio the minister.

No development or discovery will be corporated as part of IVFA's program until approved by a Bioethics Commi which, in the manner of the Wa committee and the Standing Review Advisory Committee, is likely to consis lawyers, philosophers, social workers medical researchers. The company will for the time being have its own research development function; the Monash Uni sity team, however, will continue to p lish its research results. Where patenta discoveries emerge, care will be taker enable proprietary rights to be secured l under the agreement, patenting of med procedures will not be permitted. The u versity has no equity in the company will receive royalties. Members of the l team in Melbourne will remain employ of the university and may receive paym on a consulting basis.

Professor Wood says that the money research generated by the royalty paym has become necessary to keep the team gether, given the low support accorde the IVF programme in the past; ethical jections to profit derived from the vanced techniques would, if taken appear to apply to all private medic There could be no absolute guarantee says, that a US doctor trained in the r techniques would not start his own cl offering procedures illegal in Victoria, such a clinic would then not continue to joy support from the Melbourne IVF te and a personal vetting scheme would help to minimize this risk. **Jeffrey Se**

Hetero-solid growth

UK seeks to catch up

A MULTI-MILLION pound stimulus to British research in "low dimensional structures" over the next five years has been announced by the Science and Engineering Research Council (SERC). The programme is intended to enhance the United Kingdom's ability to compete scientifically and, in the longer term, industrially in the field of heterojunction semiconductors, and the new physics associated with such devices. SERC has already allocated £3.5 million to the scheme, which forms a part of the strategy for "core science" heralded by the council's Science Board last year (see *Nature* **309**, 388; 1984).

Interest in low-dimensional structures has burgeoned over the past few years with the realization that they offer unprecedented opportunities for the study of semiconductor physics. In particular, the techniques of molecular beam epitaxy (MBE) and metal organic chemical vapour deposition (MOCVD) permit the construction of interleaved layers of semiconductor materials of precisely determined thickness — in many cases, only a few angstroms. In a superlattice, for example, layers of GaAs may alternate with GaAlAs. The difference between the two materials in their respective energy gaps separating the valence and conduction bands can result in a two-dimensional layer of highly mobile electrons. The physics of such layers, in which unique phenomena such as fractional quantum effects and phase transitions have been observed, is one of the most active research areas, while the potential industrial spin-off with, for example, high-speed devices and optical communications and logic is vast.

To a considerable extent, British researchers have, until recently, had to confine their studies to "off the shelf" materials supplied by companies such as GEC, Philips and Plessey. As a result of the new initiative, however, university centres for material growth are being established, allowing researchers not only to design and grow materials as dictated by their research but also to study and improve the growth techniques themselves. For instance, a group at the University of Cambridge, with the collaboration of GEC and British Telecom, is developing *in situ* electron lithography during growth by MBE.

MBE work will also be carried out at the University of Nottingham and Imperial College, London, while an MOCVD facility is being developed at Oxford. A typical cost for the establishment of an MBE or MOCVD facility is anything up to £500,000. Industrial collaboration will involve part-sponsored studentships and funding of cooperative research grants. In such schemes, the company involved generally has right of first refusal of patentable discoveries, proceeds being shared with the public purse through the British Technology Group.

Philip Campbell

NIH research grants

Multi-year grants on ice

Washington

THE Reagan administration's Office of Management and Budget (OMB) has struck a compromise with leaders of the Republican-controlled Senate over the number of new research grants to be awarded by the National Institutes of Health (NIH). According to the agreement, the number of such grants awarded each year would settle at around 5,500, 10 per cent above the current level but considerably below the 6,500 foreseen last year by Congress.

The agreement, if accepted on the floor of the House of Representatives and of the Senate, might defuse the confrontation with the White House over OMB's scheme to circumvent the wishes of Congress by instructing NIH to hold back $200 million of the $938 million that had been intended for this year's grants (see *Nature* **313**, 377; 1985). The issue of NIH grants remains controversial in Congress, however, and the agreement of both houses is by no means assured.

Congress, ever-generous to NIH, last year voted it a large budget increase and urged increasing the number of new and competing research grants awarded each year from 5,000 to 6,500. Because grants last typically for three years, however, Congress's action would have meant further large increases for the NIH budget in 1986 and 1987. OMB, seeking to reduce the federal budget deficit, told NIH to disregard the new grant figures suggested by Congress and to hold the number steady at 5,000; the $200 million left over as a result should be used to support second- and third-year grant continuations in 1986 and 1987.

The legal basis for OMB's action was questioned last month by the US Comptroller General, who was responding to a query by Senator Lowell Weicker. The Comptroller General's opinion was that the executive branch was not legally obliged to follow the wishes expressed in congressional appropriation committee reports about the number of grants NIH should support; on the other hand, OMB's specific plan, to fund 646 multi-year grants with the 1985 appropriation, was found to be illegal.

The administration has challenged the Comptroller General's opinion, and in the meantime the $200 million in question is being held in reserve: the matter has been referred to the Justice Department for a second opinion. As a compromise, the administration has undertaken that NIH should not commit funds to any multi-year grants before 1 July, by which time the question of legality will presumably have been sorted out.

Last week's agreement with Republican leaders refers formally only to fiscal year 1986 and beyond; it does not address the problem of what to do about this year's appropriation. It is thought likely, however, that the same figure of 5,500 new grants might be an acceptable compromise for this year as well. The administration would nevertheless still like to establish the legality of multi-year funding, despite certain opposition from Congress. Should legal obstacles prevent this, all the $200 million now being held back would have to be spent this year. One possibility being explored is that it might be used for a one-time-only extension of grants that narrowly failed to be extended this year: this would placate many disappointed researchers without incurring the commitment of a new three-year grant. **Tim Beardsley**

UK embryo research

Government stops Powell bill

The British government has now decided effectively to block the Unborn Children (Protection) Bill in its passage through the final stages of procedure in the House of Commons. The bill, which seeks to prevent the use of *in vitro* fertilized embryos for purposes other than to bring to term a particular fetus, was introduced by Mr Enoch Powell, MP, and received a large majority (127) at its second reading (see *Nature* 21 February, p.618). On 3 May, when the bill is due to be referred back to the House of Commons from committee, the government has decided not to provide enough time for debate, so that the bill's opponents will be able to talk it out. This decision has infuriated supporters of Mr Powell's bill, who argue that it should receive government backing because of the large percentage of MPs in favour of it.

A Marplan opinion poll published in Britain this week, however, reveals that 32 per cent of adults are in favour of embryo research, 41 per cent are against and 27 per cent "don't know". Nearly half of the opponents changed their minds when asked if they would favour research if it would help to eliminate genetic diseases, resulting in a total of 51 per cent in favour of research.

The government plans to introduce its own legislation, possibly in the parliamentary session beginning next October, based on the findings of the Warnock committee (see *Nature* **312**, 389; 1984). This legislation will be more comprehensive than Mr Powell's bill; Mr Norman Fowler, Secretary of State for Social Services, is pledged to prevent commercial surrogacy agencies of any kind from operating in Britain, and this will be included in the government's plans. **Maxine Clarke**

Algal structures



FIXED algae have been used for many purposes, but rarely for making what the French sculptor Ernest Pignon-Ernest calls "arbrorigènes". This pun on the French words for "tree" (arbre) and "aborigine" describes a collection of life-sized models carved out of polystyrene and containing fixed *Porphyridium cruentum*, a reddish single-celled alga that gives the polystyrene flesh tones. Pignon-Ernest's models now adorn the trees of the Paris Jardin des Plantes. The sculptor is fascinated by the idea that these sculptures need water and light.

Pignon-Ernest is not a scientist. He conceived the idea of using fixed algae, however, and approached biotechnologist Daniel Thomas of the University of Compiègne and his colleagues Claude Gudin of the solar biotechnology laboratory at Cadarache for ideas. Thomas, who specializes in cell and enzyme-fixing, had the technology of fixing P. *cruentum* (a common garden species) in polystyrene, and Gudin has been using it to produce polysaccharides using solar energy. (When its environment dries, the organism protects itself by secreting a sugary shell.) Pignon-Ernest found the material ideal, and the "breathing" arbrorigènes were the result.

Thomas, meanwhile, was also at work with another artist, Monet — or at least with his enormous "water-lilies", a 186 sq. m. canvas hanging in the Louvre gallery in Paris. This painting was damaged when it was first transported. Paper was glued to the surface of the paint to protect it, but the wrong glue was used and the picture remained misted and in danger of denaturing by interaction of glue and paint. Thomas came to the rescue, discovered the glue was starch-based and dissolved it off with enzymes. Which proves that biotechnology has its uses, even if those uses will have little effect on share prices....

Robert Walgate

Name for AIDS virus

SIR — Although I personally believe that the virus associated with acquired immune deficiency syndrome (AIDS), HTLV/LAV, has enough structural and functional similarities with the other human T-lymphotropic viruses (HTLV-I and HTLV-II) to justify its classification within the same family[1], I also recognize that more than just science and logic are at stake over the recent nomenclature debate[2]. If a more non-partisan name is ultimately called for, my personal suggestion is that the virus should be called human AIDS/lymphotropic virus (HALV). Human, because in the tradition of naming retroviruses, the species name is given; AIDS, because although it is not the only disease this virus causes, it is the one that gives it notoriety; lymphotropic, because this is the most basic and neutral characteristic of the virus. Since it looks enough like HTLV, and contains all three letters of LAV, it may well be the perfect solution for the two groups to meet *halv* way.

FLOSSIE WONG-STAAL
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1. Wong-Staal, F. & Gallo, R.C. *Blood* **65**, 253-263, (1985).
2. Marx, J.L. *Science* **227**: 1449-1451, (1985).

Aid for Chile

SIR — As you probably know, we in Chile are at present in great distress. On the evening of 3 March, Santiago suffered a catastrophic earthquake. An international campaign has already started, mainly to solve the immediate needs of the several hundred thousands of homeless people. The building of our physics department, although still standing, was severely damaged, most probably beyond repair.

Science in general and physics in particular are vulnerable in developing countries and clearly more so in situations like the present one. We scientists are often looked upon with suspicion and we cannot at present expect any significant help from the authorities.

Nevertheless, we have not lost our optimism and desire to carry on with our work. After assessing the real effects of the earthquake on our building we have decided to start a campaign to raise funds in order to build up a new one. We estimate that the basic need of the thirty physicists in our department could be met by a twenty thousand square foot construction.

We would welcome any material help or advice that our colleagues in the scientific community can offer.

LINCOYÁN GONZÁLEZ
Physics Department, University of Chile, Casilla 5487, Santiago, Chile

Embryo research

SIR — I am disgusted with your call for research ideas on human embryos (*Nature* 7 March, p.11). Any researcher participating in it (and those experimenting on human tissue obtained at abortions) should be forced to read Trevor-Roper's book review "Seas of unreason" (*Nature* **313**, 407; 1985).

The primary objective for any physician has to be care for the individual patient. We must not sacrifice human individuals for a potential benefit to others. Just as we do not kill AIDS patients to prevent the infection of others, experimentation with and killing of embryos and fetuses cannot be justified by potentially interesting research results. A statement of what those benefits might be is therefore irrelevant.

If human societies ever become aware again of those hippocratic truths, many of today's researchers will be judged then as are Third Reich doctors today. And we will ask disgustedly again, in the words of Trevor-Roper: "How could a civilized nation, in the twentieth century, not only permit it but actually cooperate in it?".

CHRISTOPH BODE
Massachusetts General Hospital, Boston, Massachusetts, 02114, USA

SIR — I read the News and Views article on human embryo experimentation (*Nature* 7 March, p.11) with incredulity.

Those of us who are not wholly in agreement with Enoch Powell's bill may believe that there are circumstances in which extreme necessity may make experiments of this type allowable. But your anonymous columnist is inviting scientists to *invent* research to do upon embryos: to dream up ideas, which might not otherwise have occurred to them, for research projects which would not otherwise have taken place.

Surely the ethical dubiousness of this incitement to experiment can only strengthen public opposition to all such experiments?

R.J. BIRD
50 Highbury, Jesmond, Newcastle upon Tyne NE2 3EA, UK

Suitable treatment

SIR — In a recent leading article about the unauthorized implant of an artificial heart, *Nature* describes the stereotypes being impressed upon the surgeon and the Food and Drug Administration (FDA) as the rugged individualist and the pin-headed bureaucrat. In the same vein *Nature*'s role could be described as the aloof and bloodless intellectual. Stripped of its urbanity, what your editorial really said was: let the patient die. *Nature* admits the implant functioned, and that the proper role for future implants may indeed be to stabilize patients awaiting appropriate transplants. Your objections to its use are very thin.

The issue of informed consent seems to me to be very artificial. Informed consent had been obtained in advance for a very aggressive procedure, a transplant. Do you really feel that even in the calm period before the surgery the patient and family would have objected to the possible use of an artificial heart on a temporary basis? The issue of FDA approval would seem very abstract and irrelevant to a family who felt that the implant had a good chance of working for the short time required.

The issue of use of a non-approved device should be put into perspective. Use of drugs for purposes not approved by the FDA is common practice in the United States. A good example is xylocaine, which is approved only as an anaesthetic, but has proved very valuable in controlling certain cardiac arrhythmias. It has been used effectively and apparently safely in that mode all over the country, and no one has said a word. Physicians use it, in some cases, because it saves patients who might otherwise die. Artifical hearts have captured the public imagination and so have engendered intense scrutiny of their use. The issues are no different from those dealt with in a more flexible way every day.

The idea that the surgical team could have been prepared with an authorized back-up is ludicrous. The cost would be astronomical, and the personnel and time are not there. The fact is that FDA has authorized a procedure, the transplant, without the possibility of the implant back-up that may be needed. Placed in this impossible situation different surgeons will react in different ways. I would prefer my surgeon to be the rugged individualist not dominated by the pin-headed bureacrat. *Nature*'s aloofness, either at the bedside or funeral, would not be welcome.

EATON E. LATTMAN
The Johns Hopkins University, Department of Biophysics, School of Medicine, Baltimore, Maryland 21205, USA

Dinosaurs did well

SIR — I am a layman, but I am tired of hearing about "theories" (really mere conjectures, as they cannot be tested) of the extinction of the dinosaurs.

They ruled the roost for a hundred million years, ample time for the opposition to get its act together, for the evolution of small, elusive animals that destroyed vegetation and ate the dinosaurs' eggs so that they died of hunger faster than they could reproduce.

They died out "suddenly" did they? How can we know? For any traces to survive, the animal had to die in very special circumstances, so the absence of evidence proves nothing. And how many hectares of the Earth have been excavated?

This kind of nonsense may be good clean fun for the palaeontologists; but people in the media seem to think that *I* wish to hear of it. I do not.

R. C. HOPE
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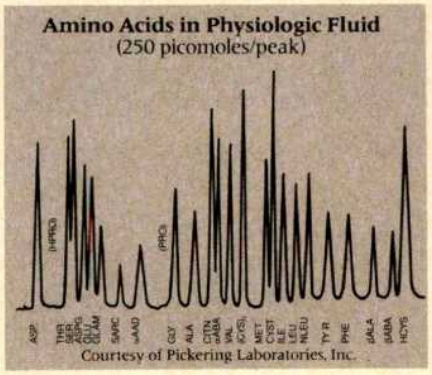
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If we need a co-ordinated policy for transport here...



While favouring the retention of the County Councils to plan and co-ordinate their own transport facilities on a regional basis, the Government is bent on abolishing the GLC.

It recognises the need for London-wide transport planning, but seeks to take this task on itself. London Transport has already been hived off to a Government-controlled quango. Now the Government proposes that the Whitehall-planned trunk road network should be extended. Whitehall will also control traffic operation on a further 300 miles of main road, and will give *mandatory* guidance on traffic schemes on all other roads.

The prime reason for change put forward by the Government – greater and more

don't we also need it here?



immediate local democracy – is, thus, a hypocritical sham. The vitally important link between public transport and traffic planning will be broken. Londoners will have no direct say in how money provided by them for transport will be spent.

Highway and traffic planning on all but the strategic network will be divided amongst 33 boroughs, so there will be no consistent policies or initiatives.

Not surprisingly, the Government has no coherent argument to support its proposals. They are recipes for jam that Londoners will have to swallow if the Abolition Bill becomes law.

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Origins of five-fold symmetry

That solids with five-fold symmetry may exist seems plausible enough, but a brief bout of calculation serves chiefly as a proof of how much more remains to be done.

LAST year's gust of excitement at the recognition of five-fold symmetry in a binary alloy (of Al and Mn) by Shechtman, Blech, Gratias and Cahn (*Phys. Rev. Lett.* **53**, 1951; 1984) has already spawned its own mini-industry. The surprise, then, was that there should be evidence of any kind for five-fold symmetry when this is strictly forbidden, on geometrical grounds, in a crystal with regular translational periodicity (see *Nature* **313**, 263; 1985).

By the end of the year, D. Levine and P.J. Steinhardt had produced an outline explanation of how such a structure could exist (*Phys. Rev. Lett.* **53**, 2477; 1984). They showed how to construct "quasi-crystalline" structures in two or three dimensions which, although devoid of translational symmetry, could have local five-fold symmetry. Now attention has turned to the question whether such structures will be energetically preferred to regular crystals. But the clutch of three papers published (in *Phys. Rev. Lett*, 8 April 1985), all of which follow the same track, show such discordancies as to suggest that the issue is far from settled.

The starting point is the Landau theory of the evolution of symmetry by means of phase transitions from, say, a liquid in the process of solidification, or one solid phase in the process of transition to another. The trick, following Landau, is to suppose that the free energy is a function of parameters which describe the degree of geometrical order in the system. The first task is to determine what order parameters are allowed by geometry.

Everybody agrees that the order parameters to use are quantities D_k which are the coefficients of mass-density waves within the aggregated system, and which turn up in mathematical expressions of the form $D_k \exp(i\mathbf{Q}_k.\mathbf{R})$, where $\mathbf{Q}_k$ is some fixed vector and $\mathbf{R}$ is the vector representing position. (The symbol i is the square root of -1.) This, of course, is a periodic function of a kind that might describe plane waves travelling in direction $\mathbf{Q}_k$.

A single order parameter, associated with a single vector $\mathbf{Q}_k$ suffices to describe the smectic phase of a liquid crystal (in which molecules assume a periodic arrangement in only one dimension within the medium in which they are dissolved). The ordering of regular crystals, on the other hand, requires at least three order parameters, each of them associated with a different vector $\mathbf{Q}_k$. These must jointly describe how it is possible to choose an infinite number of directions within the crystal in which identically constituted layers of atoms are periodically repeated (and which are defined, in crystallography, by the Miller indices *hkl*). The basic vectors describing these recurring planes of atoms are not those defining the translational symmetry of the crystal, represented by the repeated translation of the unit cell, but rather the vectors of what is called the reciprocal lattice. All this is standard textbook stuff.

The neatness of the Landau treatment stems from its representation of the free energy as a power series expansion in terms of the mass-density distribution, itself the sum of terms such as $D_k \exp(i\mathbf{Q}_k.\mathbf{R})$ taken over all possible vectors $\mathbf{Q}_k$ in the reciprocal lattice. The separate terms are themselves harmonics of similar expressions involving only the basis vectors of the reciprocal lattice.

Structures with the symmetry of a body-centred cubic lattice are thus signalled by terms which are products of six D-coefficients, each corresponding to one of the six independent vectors of the tetrahedron which is the unit structure of the reciprocal lattice. Face-centred cubic symmetry is similarly signalled by terms of the eighth power, where the corresponding vectors are those of the unit octahedra of the reciprocal lattice. Telling whether a melt will crystallize in one form rather than the other may then simply be a matter of guessing whether one form of symmetry will give a smaller free energy than the other, which in practice entails much arm-waving.

The novelty now reported is that the same argument can be used to judge the likelihood that symmetries not allowed in ordinary crystallography will occur. Per Bak from the Brookhaven National Laboratory puts the case most simply by enumerating the terms in a Landau expansion corresponding to five-fold symmetry (*Phys. Rev. Lett.* **54**, 1517; 1985). The simplest case is that of fifth-power terms (products of five D-coefficients) corresponding to the five vectors represented by the sides of an equilateral planar pentagon, but this corresponds in three dimensions merely to a structure built of parallel rods whose cross-sections are the elements of the Penrose tiling of the plane.

Bak gets true five-fold symmetry by picking out the Landau terms corresponding to the null combinations of the fifteen pairs of vectors along the edges of a regular icosahedron. These show up as ten third-degree terms and six fifth-degree terms, corresponding to the ten triangles and five pentagons that can be drawn on the surface of an icosahedron. Bak readily concludes that a structure with five-fold symmetry can be stable relative to body-centred cubic structures if only the parameters are suitably adjusted.

Levine and Steinhardt (see above), together with a number of colleagues at the University of Pennsylvania and John Toner from the IBM research centre, follow a similar tack but calculate the elastic constants of a solid with five-fold symmetry to demonstrate its stability (*Phys. Rev. Lett.* **54**, 1520; 1985). As in the simple Landau treatment, symmetry restricts the range of terms occurring in the equations, but this line of argument has the virtues of showing how dislocations may be introduced into structures with five-fold symmetry (by means of pretty pictures made by driving a dot-matrix printer by the expressions calculated for the mass-density distribution) and of making explicit the hidden symmetry of these perplexing five-fold structures, which emerge as the representation in three dimensions of much simpler structures in six dimensions.

So far, there is very little physics in these discussions, but M.D. Mermin and Sandra M. Troian from Cornell University have a novel twist (*Phys. Rev. Lett.* **54**, 1524; 1985). They say that they find only metastable states for a solid with one component and with five-fold symmetry (but they appear not to have included fifth-degree terms, corresponding to the six pentagons on the surface of an icosahedron, in their Landau calculation). But how relevant is it that the only real solid in which five-fold symmetry has been found so far is an alloy, with two components?

In an alloy, there is scope for a dimension of disorder beyond those specified by the wave vectors of the simple Landau calculation. On this view, the argument goes, there is scope for generating five-fold symmetry from a blend of geometrical shape and occupancy by one or other of two components. So far as it goes, the argument is suggestive only, although the most telling evidence is a pair of dot-matrix diagrams, said to represent a cross-section from a structure with five-fold symmetry, in which one component almost exactly fills the gaps in the distribution of the other. Time, and some tangible experimental investigation, will no doubt tell whether this is what five-fold symmetry is like.

John Maddox

Molecular Egyptology

Mummified human DNA cloned

from J.S. Jones

ON page 644 of this issue, Svante Paabo reports the remarkable extraction and cloning of DNA from an Egyptian mummy[1]. One clone contains a 3.4 kilobase segment of DNA that has been preserved for more than two millenia. Although only one out of 23 mummies has yet produced clonable DNA, the yield from this individual approaches five per cent of that obtainable from fresh human material. Preliminary though it is, the report opens up some fascinating possibilities for future work on human evolution and archaeology.

'Fossil genes' have been identified before now: ancient snails buried in prehistoric English earthworks retain their shell colour and banding patterns[2], and comparisons of skeletal variants in living and extinct mice have been used to establish the origins of some Scottish mouse populations[3]. Even mummies retain enough of their blood group substances to allow the kinship of family groups to be confirmed serologically[4] and, on the basis of hair characters, an unidentified royal mummy from the New Kingdom has been shown to be the mother of the heretic pharaoh Akhenaton[5].

Valuable as such specific data may be, there is much more potential information in DNA sequences. The human genome possesses hundreds of thousands of genetic polymorphisms at the DNA level. Some regions (which involve variations in the number of copies of repeated sequences not dissimilar to the *Alu* sequence, to which the mummy 3.4 kilobase clone is closely related) are hypervariable[6] and can provide particularly precise estimates of human kinship and population relatedness. If these polymorphisms can be detected in mummies, we may answer some important questions about the world of our ancestors.

Information on DNA sequences is uniquely suited for tracing population movements: knowledge of the geographical distribution of point mutations in some senses provides no more than single letters in a population's genetic identity, while the ordered sequence of bases provided by its DNA haplotype gives the genetic surname itself. Thus, information on DNA sequences closely linked to the sickle-cell haemoglobin polymorphism has recently been used to establish the relationships between living populations in different parts of Africa[7]. Sickle-cell populations from Senegal and Benin differ considerably in their sickle-cell haplotype; but populations from North Africa have the same haplotype as the Nigerians. This is strong evidence that the sickle-cell mutation has arisen several times, but that the northern sickling populations originated by migration from West Africa.

Information of this kind from mummies might tell us something about population movements in ancient Africa. Ancient Egypt is thought to have been occupied by a number of distinct populations, and it is certainly the case that some dynasties of Pharaohs were drawn from foreign populations that invaded the Nile Valley. Perhaps it would be possible to test the claim of the Coptic Christians of modern Egypt to be the lineal descendants of the ancients (who disappeared from the Nile Valley under successive waves of invasion by Syrians, Greeks, Romans, Byzantines and Arabs).

DNA sequences are also useful in studying patterns of kinship and paternity within local populations. There is considerable controversy among Egyptologists about the relatedness of groups of mummies from particular sites, and there is clear potential for DNA work here. Ancient records suggest that in the 18th Dynasty, the Egyptian royal family practised incest between brother and sister or father and daughter for at least eight generations: information on their mummified DNA sequences might tell us to what extent this royal exclusivity was successful. Incest in Egypt was designed to preserve the blood line of a deity. There is something intriguing about the possiblity of learning the genotype of a god.

Mummies are not unique to Egypt: preserved humans have been found in Peru, Japan, Australasia and Europe. There is the potential here to answer some larger questions abut human evolution. What is the relationship between the native Australians and the rest of the world; were there indeed two separate invasions of Australia, as often claimed? Do the genotypes of mummified Europeans accord with the suggestion that geographical patterns of gene-frequency change in modern Europeans reflect an invasion from the East that occurred as a result of the development of agriculture? Mr Average, as defined by the population nearest the global mean for a large number of enzyme and blood-group loci, lives in the Middle East[8]: would the patterns of change in mummified humans support the idea of worldwide spread from the Middle East? An answer to any of these questions would be of extraordinary interest.

Finally, it is important to understand that Pääbo has *not* done: we cannot of course reconstitute a functional gene (let alone a living individual) from this short repeated sequence. One American newspaper reported the recent cloning of mitochondrial DNA from a specimen of the extinct quagga[9] as "US Scientists Clone Dinosaurs to Fight on after Nuclear War", and probably there will be similar sensational accounts of mummy DNA. Molecular biology rarely lives up to its headlines, but the interaction of DNA technology and archaeology may open a new phase in our understanding of human history. □

1. Paabo, S *Nature* **314**, (1985)
2. Cain, A J in *Ecological Genetics and Evolution* (ed Creed, R) 65 (Blackwell, Oxford, 1971)
3. Berry, R J *Symp zool Soc Lond* **26**, 3 (1970)
4. Harrison, R G *et al Nature* **224**, 325 (1969)
5. Harris, J E *et al. Science* **200**, 1149 (1978)
6. Jeffreys, A.J , Wilson, V & Thein, S L *Nature* **314**, 67 (1985)
7. Pagnier, A J *et al Proc natn Acad Sci U S A* **81**, 1771 (1984)
8. Piazza, A A *et al Proc natn Acad Sci U S A* **78**, 2638 (1981)
9. Higuchi, R *et al Nature* **312**, 282 (1984)

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Archaeoastronomy

Halley's comet in Babylonia

from C.B.F. Walker

IN August 1984, Halley's comet was unexpectedly discovered in the British Museum or, to be more precise, on some Babylonian tablets in the museum's archives. That discovery, reported by F.R. Stephenson, K.K.C. Yau and H. Hunger on page 587 of this issue, will be exciting both for eager Halley watchers and for historians of science. It represents the first significant addition to our knowledge of the past history of the comet since the French publication of Chinese observations in 1846. It fills an important gap in the historical record, since no other account of the return of the comet in 164 BC survives. Remarkably, it also proves that the Babylonian astronomers kept records of such accuracy that we can now make a clear choice between current theories of the comet's path in antiquity.

What were the Babylonians doing recording comets? The beginnings of astronomy in Mesopotamia probably go back to the third millennium BC, when the Sumerians gave the planets and principal constellations their names but, apart from simple tables of the length of daylight and the rising and setting of constellations, almost all the early texts are concerned with astrology rather than astronomy. Writing in about AD 150, the Greek astronomer Ptolemy notes that the earliest observations available to him date from the time of the Babylonian king Nabonassar (Nabu-nasir, 747-734 BC). Although contemporary texts do not survive, later Babylonian texts also suggest that the practice of keeping a regular official record on the movements



Babylonian cuneiform tablet (BMA 41462) containing a record of Halley's comet in 164 BC (Photograph courtesy of The British Museum).

of the Moon and planets began in Nabonassar's time. The surviving texts from Babylon number rather more than 2,000, most of which are now in the British Museum. Among these, some 1,200 are fragments of observation records, a type known as 'astronomical diaries', mostly datable to between about 380 and 40 BC. Others are of a strictly mathematical character, empherides for the moon and planets. The latest text that can be dated is an almanac for AD 75.

The astronomical diaries give a day-by-day account of lunar and planetary observations and weather conditions, with monthly summaries of the height of the Euphrates, prices of various staple commodities, political events, and occasional references to comets and earthquakes, the basis for detailed mathematical systems for predicting recurrent phenomena.

The fundamental concern of the Babylonian astronomers was with the movements of the Moon and planets about the ecliptic. The fact that they moved in a precisely defined belt of stars was recognized early on, and the constellations through which they moved (at first eighteen, later reduced to twelve) were already listed in about 1,000 BC. There is a curious dichotomy between observation and theory in Babylonian astronomy. Texts of observations record the movements of the Moon and planets with reference to a list of 31 stars of varying brightness lying at unequal intervals along or near the ecliptic; distances from the 31 stars are measured in cubits (kuš), which appear to have a variable length of either 2 or 2½ degrees. The theoretical texts, ephemerides and almanacs, which first appear late in the fourth century BC, use the familiar zodiacal system of twelve equal constellations, each divided into 30 degrees (uš).

This dichotomy is also reflected in the astronomical diaries. Although these ostensibly record observations, analysis shows that all phenomena such as the equinoxes and solstices, the appearances of Sirius, and the stationary positions of planets are in almost every case derived from theoretical schemes rather than from observations.

Our primary source for the identification of the constellations is a text known as Mul-Apin, the 'Plough'. This lists the stars in various groups or 'paths' with details of their heliacal risings and settings. Other texts give descriptions of the appearance of the constellations, and there is also a very small group of drawings. At least in the zodiac belt, the constellations correspond largely to the picture familiar from the detailed descriptions of Ptolemy, but there are some interesting differences. For instance, Aries is not a ram but a 'hired worker'. More significantly, and still not fully explained, the zodiacal system does not start at the First Point of Aries, 0° ecliptic longitude, but at about 355°, a difference that extends through the whole zodiac.

In texts of observations, measurements of latitude are expressed as so many cubits 'above' (to the north) or 'below' (to the south), relative to a star or planet. Only in the lunar ephemerides is there a concept of absolute ecliptic latitude.

For chronology, the astronomical texts use the king's regnal year (or, from 311 BC onwards, the Seleucid era), the lunar month — beginning with the first sighting of the new moon — and the day. The Babylonian day began at sunset and, for astronomical purposes, day and night were divided into three watches each. Water clocks were used to time observations, the units of time being the bēru (2 hours) and the uš (four minutes). A group of 26 stars culminating at the zenith provides an additional reference point.

Edmund Halley, in the first publication of his discovery of the orbits of comets, begins by referring to a story of the classical historian Diodorus Siculus that the Chaldaeans (Babylonians) kept records of storms, earthquakes and comets, and tried to predict their occurrence. He dismisses the story as being of interest only to astrologers. Even thirty years ago we had little idea of how well informed Diodorus really was. Halley would been astonished to know how valuable the Babylonian records of comets have come to be for the study of his own comet. □

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Biotechnology

New route to antibiotic creation

from Arnold L. Demain

ALTHOUGH developments in recombinant DNA (rDNA) technology have not escaped the attention of the antibiotic industry, the report on page 642 of this issue from a collaboration of British, Japanese and American laboratories that novel antibiotics can be obtained using the technique is a major step forward in biotechnology[1].

The rapid movement of rDNA technology into secondary metabolism can be mainly attributed to the long-term dedication of David Hopwood and his colleagues at John Innes Institute in Norwich to the genetics of *Streptomyces* species. Having developed plasmid and phage vectors for applying rDNA technology to these filamentous bacteria, in 1983 the Hopwood group cloned the gene of the methyltransferase of undercylprodigiosin biosynthesis in *Streptomyces coelicolor*. (Prodigiosins are pigmented antibiotics produced by unicellular and filamentous bacteria). Independently, Williams and colleagues in Houston cloned and expressed prodigiosin biosynthetic genes of *Serratia marcescens* in *Escherichia coli*[3]. The next developments were the cloning of the *Streptomyces griseus p*-aminobenzoic acid synthase gene of candicidin biosynthesis in *Streptomyces lividans*[4] at Norwich and that of a gene of clavulanic acid biosynthesis from *Streptomyces clavuligerus* at the Beecham Laboratories[5]. Finally, the Hopwood group accomplished the cloning of a segment of chromosomal DNA from *S. coelicolor* that carries the complete information necessary for the biosynthesis of the antibiotic actinorhodin[6]. Also of interest is the recent cloning of a *S. coelicolor* regulatory gene controlling the synthesis of actinorhodin, undecylprodigiosin and A-factor (a differentiation effector in streptomycetes)[7].

In order to enhance the probability of success in their attempts to produce hybrid antibiotics, Hopwood *et al.* employed microbial cultures producing three pigmented antibiotics of the isochromanequinone class. The cultured organisms were *Streptomyces coelicolor* A3 (2) (which produces the antibiotic actinorhodin), *Streptomyces* sp. AM-716 (medermycin) and *Streptomyces violaceoruber* Tü22 (granaticin). Two hybrid antibiotics — mederrhodins A

and B — were produced following the introduction of *S. coelicolor* actinorhodin biosynthetic genes (originally located on a chromosome, but placed on a plasmid vector) into *Streptomyces* sp. AM-7161. Mederrhodin A was identified as hydroxymedermycin. Cloning of *S. coelicolor* DNA in *S. violaceoruber* yielded a third hybrid antibiotic, dihydrogranatirhodin, which was found to be the C-3 or C-1 epimer of dihydrogranaticin. Evidence is put forth that each of the novel products are produced by genetic contributions from both recipient and donor microorganisms rather than merely by activation of silent genes of the recipient.

It should be noted that this is not the first technique developed for the production of hybrid antibiotics. They have been produced by 'mutational biosynthesis' for about 15 years[8]. In this technique, an antibiotic-producing strain is first mutated so that it is blocked in forming a moiety of the antibiotic and is then fed with moieties of other antibiotics. The technique has yielded hundreds of novel antibiotics but unfortunately none has become commercially important. A second method has involved natural mating between different actinomycete strains or species[9-11], and seems to produce new antibiotics by activation of latent genes in the recipient strain[12]. A third method involves protoplast fusion between strains producing different antibiotics[13].

Since new antibiotics are going to be required for many years to come in order to counteract the development of resistance in infectious microbes and the existence of diseases still unconquered by available compounds, antibiotic creation by rDNA technology is a welcome addition to existing techniques. But in case any group jumps to the erroneous conclusion that conventional efforts are now superceded, it should be understood that the rDNA technique is only a tool to be added to those of soil-screening, mutational biosynthesis and protoplast fusion. In our continuing struggle against infectious disease every new technique is welcome. □

1 Hopwood, D A *et al Nature* **314**, 642 (1985)
2 Feitelson, J S & Hopwood, D A *Molec gen Genet* **190**, 394 (1983)
3 Dauenhauer, S A, Hull, R A & Wilhams, R P *J Bacteriol* **158**, 1126 (1984)
4 Gil, J A & Hopwood, D A *Gene* **25**, 119 (1983)
5 Bailey, C R, Butler, M J, Normansell, N D, Rowlands, R T & Winstanley, D J *Bio/Technology* **2**, 808 (1984)
6 Malpartida, F & Hopwood, D A *Nature* **309**, 462 (1984)
7 Horinouchi, S, Hara, O & Beppu, T *J Bacteriol* **155**, 1238 (1983)
8 Daum, S & Lemke, J *A Rev Microbiol* **33**, 241 (1979)
9 Ihn, W, Schlegel, B, Fleck, W F & Sedmera, P *J Antibiot* **33**, 1457 (1980)
10 Mazieres, N, Peyre, M & Penasse, L *J Antibiot* **34**, 544 (1981)
11 Traxler, P, Schupp, T, Fuhrer, H & Richter, W J *J Antibiot* **34**, 971 (1981)
12 Hopwood, D A in *Biochemistry and Genetic Regulation of Commercially Important Antibiotics* (ed Vining, L C) 1 (Addison-Wesley, Reading, Massachusetts, 1983)
13 Ikeda, H, Inoue, M, Tanaka, H & Omura, S *J Antibiot* **37**, 1224 (1984)

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Oceanography

Residence times of water masses in regions of the ocean

from John Woods

WHEN a water molecule involved in the global hydrological cycle finds itself in the atmosphere, it is likely to reside there for about ten days. The residence times of water in other components of the hydrological cycle are approximately: Southern Sea ice, a few months; Northern Sea ice, a few years; the World ocean, 3,000 yr; lakes, 100 yr; and the polar glaciers, tens of millenia. These estimates are based either on the ratio of volume-to-surface flux for each component, or on the radioactive age of materials mingled in with the water. There are errors in the estimates, but there is no fundamental difficulty in determining residence times for such distinct components of the hydrological system as the Greenland ice sheet, or the Mediterranean Sea. But problems arise when one tries to subdivide the components further, to discover whether water resides much longer in some parts than others. This is a question of fundamental importance for oceanography, but remains beset with confusion. The problem is first how best to delineate the separate parts and then how to determine their residence times. Equivalent questions arise in systems that range from the cardiovascular system to chemical reactors. The letter by Buffham[1] on page 606 of this issue helps to clarify matters.

Buffham's contribution is to introduce a formalism into the analysis of what he calls "region residence times" of complex circulation systems. The idea is deceptively simple and with hindsight one is tempted to dismiss it as obvious. Yet it is far from trivial and will influence, for example, the way oceanographers study their complex circulation system. What Buffham shows is that the region residence time can be deduced from a model of the system if the volumes of all regions of the model other than the one of interest are reduced to zero and by finding the residence time distribution of the resulting model. He shows that this approach is consistent with previous results and illustrates it with some simple configurations.

The method has potential for the diagnosis of region residence times in models of ocean circulation. A good example of the need for such information is provided by the problem of trying to predict climatic responses to atmospheric pollution by CO_2. Pollution decreases the net infrared cooling of the ocean, which therefore accumulates heat until the surface temperature of the ocean rises sufficiently to rebalance the surface heat budget by enhanced conduction and evaporation. The oceanographer's problem is to predict the global pattern of increasing surface temperature. For that, it is necessary to calculate the distribution of the heat anomaly created by the greenhouse effect. Early models assumed that the anomaly stayed at the surface in a mixed layer 70 metres deep. This assumption produces a characteristic (e-folding) response time of a few years. There are, however, theoretical objections to such models because they neglect the ventilation of anomalies from the mixed layer into the permanent thermocline[2]; models that include this factor give response times of several decades.

Which, if any, of these predictions is correct could be of great importance for the way that society prepares itself for changes in sea surface temperature, and hence global climate, in response to CO_2 pollution. A slow change would give time to switch to nuclear power, adapt agriculture, build up sea defences, even to move vulnerable populations. If the ventilation of the deep ocean is a factor, then the response may be so attenuated that we can enjoy the agriculture benefits of increased CO_2 production without any unpleasant change in climate. To predict the response accurately requires that the models used have the correct residence times in all the regions through which the thermal anomaly passes. Vigorous efforts are being made to test the ocean models by comparing the distributions of transient tracers in the ocean with those simulated by the models[3]. The problem is to diagnose the sources of differences, in particular to identify regions in the model that have unrealistic residence times.

That is where Buffham's formalism suggests a research strategy. Of course, ocean circulation is extremely complex and far from being fully understood, but we do know what goes into the models, and sensitivity studies related to residence time can help us to focus on the main issues. Various methods have been proposed for dividing the ocean into layers (for example, Wüst's Kernschicht-Method[4] and Worthington's isothermal surfaces[5]) but it is now generally accepted that the best division is by isopycnic (constant density) surfaces. Some models are constructed with an isopycnic distribution of grid points[6]. The advantage is that particles circulate around the permanent gyres and are mixed by synoptic-scale eddies along isopycnic surfaces; mixing through the surfaces is very much weaker and can sometimes be ignored. Using isopycnic surfaces ensures that the upper and lower boundaries of the (Buffham) regions can be treated as if they are impermeable, or only slightly leaky.

The lateral boundaries present greater

problems, which are the subject of lively debate in the ocean-circulation community. In general, the periphery of the region is defined by mean streamlines of the large-scale circulation, and by turbulent boundary layers where it meets the seabed or sea surface. These boundary layers tend to vary diurnally and seasonally, and with changing weather and climate. Since the flux of water into a particular region may depend critically on such variations, these have to be taken into account very carefully when calculating residence time.

The climatological problem focuses on the exchange between atmosphere and ocean that occurs through the surface boundary layer. Some regions of the ocean have peripheries that include the surface boundary layer only in winter, and some not every winter. It has been suggested that some regions of the North Atlantic have not been so connected to atmospheric influence since the little Ice Age over a century ago[5], and parts of the deep Pacific may have been sealed off since the last glaciation. The ventilation of such regions depends on isopycnic diffusion — which can be estimated accurately; intrusion from the seabed boundary layer — about which we know very little; and slow leakage through the isopycnic surfaces defining the top and bottom of the layer — about which we know even less.

Geochemical dating is vital for estimating the residence time of such regions. They do not necessarily lie only in the depths of the ocean. Theoretical studies have recently suggested they will sometimes be found in the upper thermocline, for example, in the ventilation 'shadow zone' off North-West Africa[7]. The regional residence time problem is central to oceanography. It must be solved before we can expect reliable predictions of climatic change. □

1 Buffham, B A *Nature* **314**, 606 (1985).
2 Bretherton, F P *Prog Oceanogr* **11**, 93 (1982)
3 Sarmiento, J J *Phys Oceanogr* **13**, 1924 (1983)
4 Wust, G *Meteor Expedition Report* Pt 1, Vol 2 Berlin (1936)
5. Worthington, L V *On the North Atlantic Circulation* The John Hopkins Oceanographic Studies 6 (1976)
6 Bleck, R & Boudra, D.B *J phys Oceanogr* **11**, 755 (1981)
7 Luyten, J , Pedlosky, J & Stommel, H *J phys Oceanogr* **13**, 292 (1982)

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Immunology

Immunoglobulin-related domains for cell surface recognition

from Alan F. Williams

Is it acceptable to categorize cell-surface molecules as recognition structures because they show a clear relationship to immunoglobulin (Igs)? Perhaps not, but one of two newly reported members of the Ig superfamily clearly supports the recognition theme since it is a cell-surface molecule that seems to have an important accessory function in the recognition of antigen by T lymphocytes[1,2]. The other new structure[3] is also found in the T-lymphocyte lineage and it is immediately obvious from the figure and the table that much of the surface of thymocytes and unactivated T lymphocytes is covered with molecules that are now known to be Ig-related.

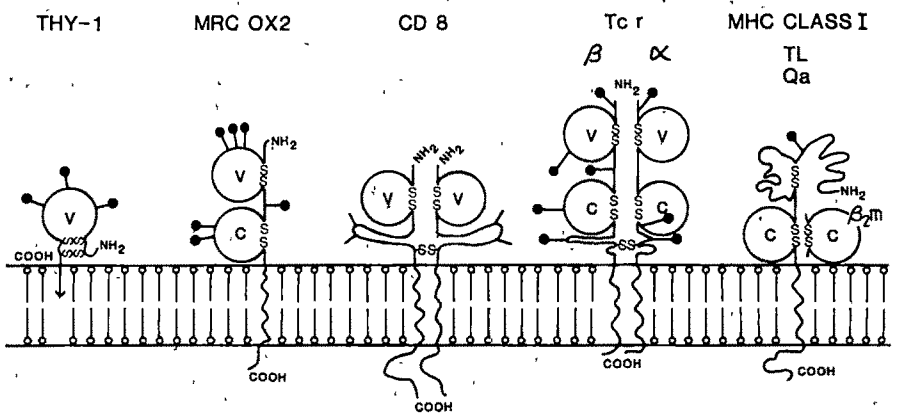
Human CD8 antigen is a newly-sequenced molecule with a role in T-lymphocyte activation[1,2]. It is also known as T8 or Leu 2, and corresponds to mouse Ly-2,3 antigen, which was one of the first four T-cell alloantigens described by mouse immunogeneticists[4]. CD8 is found on most thymocytes but amongst mature T lymphocytes it is restricted to those that are precursors for cytotoxic cells (T lymphocytes which kill, for example, virus-infected cells) which recognize, or are restricted by, class I MHC antigens and constitute about 25 per cent of recirculating T lymphocytes[5].

Antibodies to CD8 antigen can block T-cell cytotoxicity in the recognition phase of the response, so what is the role of CD8 in the cytotoxic reaction? It does not provide determinants that are complementary to foreign antigen but may interact with constant parts of class I MHC antigen to bias cytotoxic cells towards reacting with foreign antigen associated with the class I MHC structure[6]. This could be important if the cells' T-antigen receptors (Tcr), which bind foreign antigen plus MHC antigen, only recognize the polymorphic part of the latter, and so cannot distinguish class I from class II MHC structures. It should, however, be emphasized that an interaction between CD8 and class I MHC is not established and a clear demonstration of this would be a major advance.

The molcular forms of CD8 antigen are complex and vary between species. Mouse CD8 is a heterodimer consisting of an α- or α'-glycoprotein chain (M_r 38,000 and 35,000), which expresses the Ly-2 alloantigenic determinant, and a β-chain (M_r 30,000) which expresses the Ly-3 determinant[7]. Rat thymocyte CD8 also has two dissimilar chains, (M_r 38,000 and 34,000)[8] but human CD8 consists of only one chain ($M_r \sim 34{,}000$) which forms a homodimer[9], although this is further associated with a 45,000 M_r structure on thymocytes. The human antigen was cloned after transfection of mouse L cells with human DNA[1,2,10], which led to the cloning of equivalent mouse and rat antigens.

The structural features and relationships of human CD8 are summarized in the figure. Each chain contains one domain that resembles an Ig variable (V) domain, in particular an Ig light-chain V. Following this is a sequence like an Ig hinge, a transmembrane piece and a short cytoplasmic tail. The glycoprotein structure is unusual in having only *O*-linked carbohydrates[9] (there is an Asn-Pro-Thr sequence in CD8 but the proline debars *N*-glycosidic attachment at the asparagine). The larger chain of the mouse heterodimer (Ly-2) is equivalent to human CD8 (L.A. Herzenberg *et al.*, J.R. Parnes *et al.* personal communications) while, surprisingly, the equivalent in the rat is the smaller chain (P. Johnson *et al.* personal communciation). The mouse and rat chains have three and one sites for *N*-glycosylation, respectively, so differences in glycosylation may account for the species variations in size of the chains. The other CD8 glycoprotein chain of rodents remains to be cloned; its sequence will be of considerable interest to the full understanding of a set of molecules that seem to show considerable diversification in evolution.

The second new member of the Ig superfamily is the rat MRC OX-2 antigen,



Models for Ig-related molecules found on thymocytes and/or unactivated T lymphocytes. Circles indicate segments related to Ig domains with similarity to a V-domain (V) or C-domain (C) folding pattern. Double S symbols indicate possible positions of disulphide bonds but these are established only for Thy-1 and MHC class I antigens. *N*-linked carbohydrate structures (Y) are determined by positions of Asn X Thr or Ser in the protein sequence but the number of *O*-linked structures (|) in CD8 is arbitrary and the positions are guesswork. For Thy-1, see ref. 25; OX-2, ref.3; CD8, refs 1 and 2; Tcr, ref. 26; MHC class I, TL, Qa, ref. 27.

which is one of the new generation of molecules that has been discovered by means of monoclonal antibodies. This antigen was initially of interest to my colleagues and me because of its expression on thymocytes and neurones, as is the case for Thy-1 antigen[3]. OX-2 is also on all endothelial cells, B cells, activated T cells and follicular dendritic cells. There is no evidence concerning its function and no obvious functional correlation between the cells that express the antigen, except that all may be involved in recognition events. The predicted structure for OX-2, which is included in the figure, shows that it looks remarkably like a single Tcr chain or Ig light-chain with a membrane-spanning segment added. The sequence of OX-2 domain I fits well with the V-domain pattern, perhaps with a closer relationship to Thy-1 than to other sequences.

The Ig-domain hypothesis[11] says that the evolutionary starting point for Ig was a single domain which gave rise to a structure like an Ig light-chain from which all the Ig chains evolved. This concept holds good for all the molecules in the figure and it seems likely that the primordial molecules were involved in cell surface functions. It is particularly interesting that the functions of the Ig-related molecules include interactions between members of the Ig superfamily[12] (for example, Tcr and MHC; Poly Ig R and Ig A or Ig M; CD8 and MHC class I?; CD4 and MHC class II?). Such reactions may be the general rule, which suggests that the starting point was a recognition system in which Ig-like domains on one cell interacted with identical structures on another. Gene duplication and divergence could have led to interactions based on pairs of dissimilar Ig-related molecules and to the systems that now function in vertebrates.

Since the lymphocyte-based immune system has not been convincingly shown in invertebrates, it is probable that Ig-like structures first evolved on cells that had no immune function. Primitive sensory cells may have been the starting point since vertebrate neurons express Thy-1 and MRC OX-2 antigens and these seem the best candidates for contemporary structures corresponding to the postulated one- and two-domain primordial molecules. It could be argued that the CD8 structure is close to the primordial domain, except that it is most closely related in sequence to light-chain V domains rather than being apparently equidistant from the various V-like structures, as is Thy-1 antigen. Also CD8 and V-kappa genes are closely genetically linked[13] in man and mouse suggesting that they had a common immediate precursor, possibly with a two-domain structure. □

Table 1 Some surface molecules of thymocytes and unactivated T lymphocytes

Molecules	Molecular nature	Commonly-used names: Man	Mouse	Rat	Expression	Comments
Ig-related						
T-antigen receptors (Tcr)	gp43, gp40	No antibodies react with all Tcr			Thymocytes, T-helper and T-cytotoxic cells	Tcr seems the same for T-helper and T-cytotoxic cells[26]
MHC Class I	gp43; p12	HLA A,B,C	H-2 K, D	RT1A	Many cells, not brain, not 70% of thymocytes	Recognized along with foreign antigen by Tcr
TL (CD1?)	gp43, p12	NA1/34, T6	TL	NA	Thymocytes and leukaemias	Similar to and genetically linked to MHC I
Qa	gp43, p12	NA	Qa	NA	Lymphoid, others?	Similar to and genetically linked to MHC I
Thy-1	gp18	Thy-1	θ, Thy-1	Thy-1	Neuronal and fibroblasts in all species studied, lymphoid in rodents, not man	Anti-Thy-1 mitogenic for mouse T cells and affects behaviour of neurons[18]
MRC OX-2	gp47	NA	NA	OX-2	Thymocytes, neuronal, endothelium, follicular dendritic, B cells	Appears before Thy-1 in brain development[19]
CD4	gp55	T4; Leu 3	L3T4	W3/25	Thymocytes, T helpers, macrophages	Antibodies block T-helper response to antigen Homology to Ig reported in abstract[20]
CD8	gp38, gp34 or gp34×2	T8, Leu 2	Ly-2, 3	OX-8	Thymocytes, T cytotoxic, NK cells	Antibodies block T-cytotoxic cell reactions
Not Ig-related						
CD3	gp25, gp20, p20	T3, Leu 4	NA	NA	Thymocytes and mature T cells only	Associated with Tcr, antibodies mitogenic if crosslinked at surface, gp20 not Ig-related[17]
L-CA	gp180-240	L-CA	T200, Ly-5	L-CA	All leucocytes but not erythroid cells Absent from other tissues	Antibodies have functional effects Sequence shows M_r 80,000 cytoplasmic domain[21]
LSGP	gp95	L10	NA	W3/13	Thymocytes, T cells, neutrophils, plasma cells, brain	Mucin-like, peanut lectin receptor, deficiency may cause Wiskott-Aldrich syndrome[22]
LFA-1	gp180, gp90	LFA-1	LFA-1, Ly-15		Most leucocytes	gp90 shared with Mac-1; gp180 may be related to interferon[23] Antibodies inhibit T cell responses
Relationship to Ig unknown (not all antigens listed)						
CD2	gp50	T11, LFA-2	NA	NA	Thymocytes, T lymphocytes	Receptor for sheep erythrocytes Antibodies can be mitogenic[24]
CD5	gp67	T1; Leu 1	Ly-1	OX-19	Thymocytes, T lymphocytes, B-lymphocyte subset	Antibodies augment T-lymphocyte responses

Names for antigen molecules are traditional or, where possible, the new nomenclature for human antigens is used (see ref 14) Many of the data are described in ref 14 (man), refs 7 and 15 (mouse) and ref 16 (rat) Equivalent human, mouse and rat antigens are grouped where the similarities seem strong but sequence data will be needed ultimately to prove species equivalence

NA, no antibody available (but at the DNA level human OX-2 has been identified (M J Clark, G McCaughan and A N Barclay, unpublished observations) as has mouse CD3 (ref. 17)). gp, Glycoprotein, p, protein, numbers give the approximate relative molecular mass (M_r) $\times 10^{-3}$

1 Littman, D R., Thomas, Y , Maddon, P.J , Chess, L & Axel, R. *Cell* **40**, 237 (1985)
2 Sukhatme, V P , Sizer, K C , Vollmer, A C , Hunkapiller, T & Parnes, J R *Cell* **40**, 591 (1985)
3 Clark, M.J , Gagnon, J , Williams, A F & Barclay, A N *EMBO J* **4**, 113 (1985)
4 Boyse, E A &, Old, L J *A Rev Genet* **3**, 269 (1969)
5 *Immun. Rev* **74** (1983)
6 Reinherz, E L Meuer, S C & Schlossman, S F *Immun Revs* **74**, 83 (1983)
7 Walker, I D *et al Immun Rev* **82**, 47 (1984)
8 Thomas, M L & Green, J R *Eur J Immun* **13**, 855 (1983)
9 Snow, P M *et al J Immun* **133**, 2058 (1984)
10 Kavathas, P *et al Proc natn Acad Sci U S A* **81**, 7688 (1984)
11 Hill, R L *et al Proc. natn Acad Sci U S A.* **56**, 1762 (1966)
12 Williams, A F *Nature News and Views* **308**, 12 (1984)
13 Sukhatme, V P *et al J exp med* **161**, 429 (1985)
14 Bernard, A *et al Leucocyte Typing* (Springer, 1984)
15 *Immun Rev* **68** (1982)
16 Mason, D W *et al Immun Rev* **74**, 57 (1983)
17 van der Elsen, P *et al Nature* **312**, 413 (1984).
18 Leifer, D *et al. Science* **224**, 303 (1984)
19 Webb, M. & Barclay, A N *J Neurochem* **43**, 1061 (1984)
20 Littman, D R *et al ICSU Short Rep* **2**, 233 (1985)
21 Thomas, M L. *et al Cell* (in the press)
22 Remold-O'Donnell, E *et al J exp Med* **159**, 1705 (1984)
23 Springer, T A , Teplow, D B & Dreyer, W J *Nature* **314**, 540 (1985)
24 Meuer, S C *et al Cell* **36**, 897 (1984).
25 Williams, A F & Gagnon, J *Science* **216**, 696 (1982)
26 Chien, Y *et al Nature* **312**, 31 (1984)
27 Kimball, E S & Colligan, J E *Contemp Topics molec Immun* **9**, 1 (1983)

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Plant morphogenesis

Oligosaccharide floral messages?

from Keith Roberts

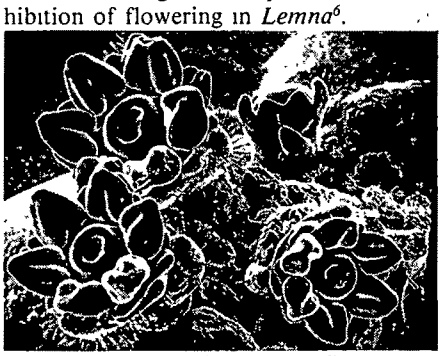
ON page 615 of this issue, Peter Albersheim and colleagues present some initial results that clearly suggest new explanatory hypotheses for the normal growth and development of plants Their general suggestion is that locked up in the bewildering array of very complex polysaccharides that form the plant cell wall there are short oligosaccharide sequences which, when released, form a set of important signalling molecules that plant cells can use in a variety of ways[1].

There are fundamental differences between the ways that plants and animals develop, most of which rest on the fact that plant cells are immobilized inside a relatively rigid cell wall. Since the wall precludes cell movement, orderly morphogenesis of plants and their organs must depend crucially on a combination of precisely positioned planes of cell division, and controlled and coordinated cell growth[2]. It is a salutory fact that we know very little either about the nature of the spatial controls, or about the molecular mechanisms involved in the determination and control of morphogenetic events. This great area of relative ignorance is all the more surprising when one considers that the plant kingdom offers wonderful experimental systems with unparalleled plasticity of developmental programmes, and that intensive research on plant growth regulators has been going on for over half-a-century.

The whole field is clearly in need of new models that can stimulate fresh approaches to the problem, and the suggestion that oligosaccharides are regulatory molecules provides a good start. The particular experiments reported on page 615, which demonstrate that artificially produced cell wall fragments can switch the experimentally induced pathway of morphogenesis in a model plant system, should be seen in the context of the more general hypothesis.

In the course of chemical work describing the variety of polysaccharides in the primary cell wall, Albersheim and his group found that oligosaccharides released in very low concentrations from the wall of the fungal pathogen *Phytophthora* triggers the expression of plant genes in a response that leads the plant to produce an antibiotic, or phytoalexin[3,4]. (Oligosaccharides that induce plants to produce phytoalexins are called elicitors and their role in stress and pathogenicity has been discussed recently in these columns[5]) It was later found that fragments of the plant cell wall, or endogenous elicitors, might also be involved in the response This fact, linked with the general realization that signalling molecules in plants are probably small water-soluble molecules that seem not to include the peptide and amino acid derivatives used in animal cell signalling, led to the idea that cell wall oligosaccharides might be used more generally by plants as signalling ligands.

Albersheim coined the term oligosaccharins for regulatory molecules that are derived from plant cell wall polymers. Those that have been described so far are 7-15 sugar residues long and have all been obtained either by chemical or enzymatic degradation of the pectic or hemicellulosic fractions of the cell wall. The range of biological effects that oligosaccharins can elicit is wide and includes the inhibition of auxin-induced growth in peas and the inhibition of flowering in *Lemna*[6].



Scanning electron micrograph of flowers on a tobacco explant, each with an ovary surrounded by stamens (Courtesy L Jehanno and K Tran Thanh Van)

The new data, which considerably extend the 'oligosaccharin hypothesis' to include a possible regulatory role for oligosaccharins in normal organogenesis, come from a colloboration between Albersheim's group and that of K. Tran Thanh Van. In her elegant system of controlled morphogenesis in thin cell-layer explants of tobacco, controlled combinations of auxin, cytokinin, and *p*H are used to dissect the regeneration of roots, vegetative buds, floral buds or callus on the explants[7]. In the present experiments sets of oligosaccharides, one prepared chemically and the other enzymatically from cell-wall pectin, are shown to switch the expected pathway of morphogenesis at concentrations estimated to be no more than 10^{-8}-10^{-9} M. Thus, with one of the preparations, flower buds can be switched to vegetative buds at *p*H 3.8, but at *p*H 6.0 the switch is in the opposite direction. With the other preparation, flowers give way to vegetative buds at *p*H 3.8, roots are induced instead of only callus at *p*H 5.0, and at *p*H 6.0 calus and vegetative buds become callus and roots.

Although neither the chemical identity of the oligosaccharins involved nor any hints as to their mode of action are yet known, the effects themselves are very dramatic and immediately pose exciting questions about the possible role of the oligosaccharins *in vivo* and in the practical context of plant regeneration. The area of work in plant development opened up by oligosaccharin probes is enormous, and already collaborations are in hand to examine the changes in gene expression induced by them in this particular system.

What these experiments throw into very clear focus is our ignorance of the molecular events involved in cell signalling and recognition mechanisms in plants. As yet, know next to nothing about cell surface receptors or signal transduction. The tobacco explant system, with its interaction of hormones, sugar and *p*H, may be too complex for comfort, but the oligosaccharin hypothesis certainly opens the way to a molecular understanding not only of plant morphogenesis but also of plant cell receptors and signalling mechanisms in general. □

1 Tran Thanh Van, K *et al Nature* **314**, 615 (1985)
2 *The Cell Surface in Plant Growth and Development* (ed Roberts, K *et al*) *J Cell Sci* suppl 1 (in the press)
3 Darvill, A G & Albersheim, P *Ann Rev Plant Physiol* **35**, 243 (1984)
4 McNeil, M *et al Ann Rev Biochem* **53**, 625 (1984)
5 Cullis, C *Nature News and Views* **310**, 366 (1984)
6 Darvill, A G *et al* in *The Cell Surface in Plant Growth and Development* (ed Roberts, K *et al*) *J Cell Sci* suppl 1 (in the press)
7 Tran Thanh Van, K *Ann rev Plant Physiol* **32**, 291 (1981)

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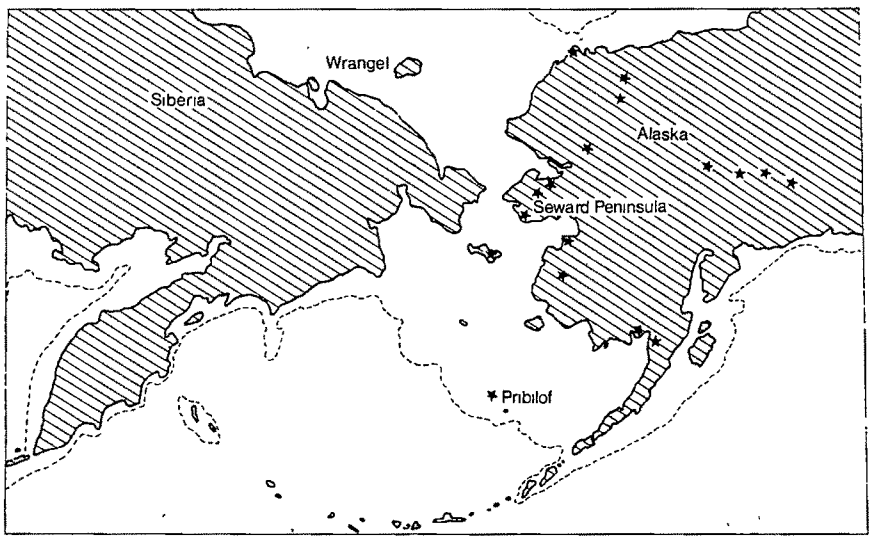
Early Americans

Land bridge of Duvanny Yar

from Paul A Colinvaux

PRESENT uncertainties about the history of the Bering land bridge and hence of the first peopling of the Americas seem close to resolution. The debate among Siberian geologists is being settled in favour of the modern interpretation, closely associated with Academian N.A. Shilo, who visited Alaska last summer and discussed with a US team what studies would be necessary in Siberia and Alaska to resolve many of the remaining uncertainties.

In the last glaciation, the Bering land bridge fused Siberia to Alaska as an ice-free plain of continental proportions (see figure). Archaeologists who claim that people lived in America more than 20,000 years ago require the land bridge to have been inhabited early in glacial times; those who dismiss all evidence for pre-Clovis peoples in the New World are content for the first inhabitants of the land bridge to have been in place only in the late glacial, shortly before melting glaciers flooded their new homeland. Either way, the inhabitants orig-



Map of the Bering Strait study area. The broken line indicates the Bering land bridge and asterisks mark the sites of the pollen analyses that have been made. Will they be matched in Siberia?

inated in Siberia, where people are known to have lived as hunters at the forest steppe ecotone over thirty thousand years ago.

The contribution of Shilo and his followers has been to define the geomorphology of north-east Siberia so explicitly that firm environmental limits for the land bridge in full glacial times are established. In eastern Siberia, large rivers flow north and are separated by interfluves that are hundreds of kilometres wide. The surface of the interfluves has a topography of flat hillocks and depressions called *yedoma* and *alasy*. It has been evident for some time that the *alasy* depressions are thermokarst features melted out of a once uniform plain of *yedoma* silt.

For many years, Siberian geologists identified the *yedoma* deposits as alluvium and suggested that there had been huge shallow lakes in which the silt settled. Shilo came to question this view during his many years in Siberia, working on gold placers (as a geomorphologist and as a student of frozen mammoth carcasses), before he became head of the Far-Eastern Science Research Centre in Vladivostok[1]. He argued that the *yedoma* silt was not alluvium, but loess. This hypothesis has been further developed by Boris Yurtsev[2] and S.V. Tomirdiaro[3], who have marshalled the data of palaeobotany, mineralogy and geomorphology until the argument for a loessic origin for the *yedoma* has become compelling: Thus older Russian descriptions of Beringian environments are turned on their heads, with 'dry, cold and windswept' substituted for the 'wet and flooded'; it now seems that the silt was blown over an arctic landscape and frozen into place. The dry, cold time of the loess accompanied the Duvanny Yar (Wurm-Wisconsin) glaciation in Siberia. The cutting of *alasy* depressions by thermokarst is now dated, by radiocarbon, to the Holocene.

D.M. Hopkins, the US authority on the Pleistocene geology of Bering Strait[4], who travelled to some of the Siberian sites with Yurtsev in 1981, agrees with the interpretation of the Shilo school. The *yedoma* is comparable to the 'muck' of the Yukon gold placers, and to the thick loess mantle on the central Seward Peninsula[5]. Hopkins also finds evidence for sand, as well as loess, blowing across Alaska, from deep in the interior down to the south land-bridge coast at the Pribilof Islands (see figure).

This view of the land bridge of Duvanny Yar times as dry, cold and barren has also been developed independently from the evidence of pollen in lake sediments. Pollen spectra dated to the land bridge on the Alaskan side represent a tundra that has no exact modern analogue and probably had a less complete vegetation cover than does modern Alaska. The extreme view is that of Ritchie and Cwynar[6], who use the first good pollen influx data to argue that the land-bridge tundra was as sparse as polar desert. Other pollen analysts argue for something like a dry facies of modern tundras but with fewer sedge meadows and almost devoid of dwarf shrubs[7,8]. In any case, pollen data seem quite incompatible with a more productive vegetation, like steppe or grassland. When Yurtsev talks of the 'tundra-steppe' that covered the *yedoma* as it formed, he likens this to the sparse tundra of modern Wrangel Island in the Arctic Ocean, his ideas thus converging with the conclusions of pollen analysts on the Alaskan side[2].

This reconstruction is in direct conflict with the hypothesis that the Duvanny Yar land bridge supported a complex biocoenosis of big game animals and, hence, a considerable production of plants — the so-called 'mammoth steppe'[8-10]. Archaeologists who argue for early peopling of the New World rely on the availability of considerable populations of mammoths and other game as the resource of their first Americans[11,12]. If the Shilo school and the Alaskan pollen analysts interpret the Duvanny Yar ecosystem correctly, it is difficult to see how the necessary population of big game could have been supported. In this event, interest would turn to the late glacial land bridge, when changing climate could have made the southern land-bridge coast more hospitable, just in time for the immediate ancestors of the Clovis culture to supply the first Americans.

As accelerator-dating increasingly brings into question claims of early American sites of human habitation[13], the idea of a frigid barrier of a land bridge, more than 14,000 years ago, becomes even more appealing. But obviously this reconstruction requires further testing and an important test will be found in applying pollen analysis to lake sediments of the Siberian side. With that in mind, the Climate Dynamics and Polar Earth Sciences programmes of the US National Science Foundation have provided funds for a team from the University of Washington and Ohio State University to work with Academician Shilo and his group on raising sediment cores from a series of lakes in Soviet Beringia. The US team will take their rubber boats and Livingstone sampling equipment, closely modelled on the equipment first introduced into palaeoecology for use in Alaskan studies[14]. The two teams plan to use this equipment in Siberia later this year to raise duplicate cores from each lake, and thereafter to conduct pollen and other palaeoecological analyses in parallel.

East and West are divided at the Bering Strait, but we propose to standardize research methods for a final reconstruction of the years when the Strait was abolished and the continents were fused. □

1. Shilo, N A *Tr Inst Magadan Akad Nauk SSSR* **11**, 154 (1964)
2 Yurtsev, B A *Paleoecology of Beringia* (ed Hopkins, D M *et al*) 157 (Academic, 1982)
3 Tomirdiaro, S V *Paleoecology of Beringia* (ed Hopkins, D M *et al* (Academic, 1982)
4 Hopkins, D M *Science* **129**, 1519 (1959)
5 Hopkins, D M *Paleoecology of Beringia* (ed Hopkins, D M *et al*) 1 (Academic, 1982)
6 Ritchie, J C Cwynar, L C *Paleoecology of Beringia* (ed Hopkins, D M *et al*) 113 (Academic, 1982)
7 Ager, T A *Paleoecology of Beringia* (ed Hopkins, D M *et al*) 75 (Academic, 1982)
8 Colinvaux P & West, F *Q Rev Archaeol* **5**, 10 (1984)
9 Matthews, J V *Am Quat Ass Abstracts* 4, 73 (1976).
10 Guthrie, R D *Paleoecology of Beringia* (ed Hopkins, D M *et al*) 307 (Academic, 1982)
11 Morlan, R E *Archaeol Survey Canada Pap* No 94 (1980)
12 West, F H *Q Rev Archaeol* 4, 13 (1982)
13 Bada, J L *et al Nature* **312**, 442 (1984)
14 Livingstone, D A *Ecology* **36**, 137 (1955)

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100 Years Ago

Far-sightedness

IN THE spring of 1837 I was travelling from Rome, northwards, by 'Vetturino,' and from the summit of Apennine on the road between Florence and Bologne, I saw, with astonishment, the whole range of the Swiss Alps, not merely distinguishable but conspicuous. Measured on the map in a direct line the nearest part of the range was distant about 200 miles The extreme portions, including Mont Blanc, were considerably more. I have no doubt that the atmospheric conditions were unusually favourable As a peasant, living on the spot, shortly passed, I asked him what mountains they were — to which he immediately answered to my surprise, that they were the mountains of Switzerland.

J HIPPISLEY

From *Nature* **31** 553, 16 April 1885

Molecular biology

Reverse transcriptase rides again

from Harold E. Varmus

JUST fifteen years ago an explosive growth in RNA tumor virology was triggered by the discovery of reverse transcriptase[1,2] — the enzyme of retroviruses that copies their RNA genome into DNA, which can then become integrated as a 'provirus' into the DNA genome of infected cells. Yet reverse transcriptase in recent years seems to have given up centre stage to oncogenes and proto-oncogenes. If so, there is now furious activity in the wings: recent findings about the *pol* genes of retroviruses — and about related genes in other viruses and in transposable elements of flies and yeast — signal a new era of discovery likely to influence such diverse topics as mechanisms of transposition, molecular evolution, and translational fidelity.

Pol coding domains

What has come to be called a retroviral *pol* gene consists of a large open reading frame, situated between reading frames for core (*gag*) and envelope (*env*) polyproteins, that encodes a protein with both reverse transcriptase RNA-directed DNA polymerase and ribonuclease H activities[3] (see the figure). Most retroviruses express *pol* by first synthesizing a *gag-pol* polyprotein (discussed further below) that is subsequently cleaved to mature components, including a virus-associated DNA endonuclease[4-6], most likely by a virus-encoded site-specific protease[7-9].

Structural studies of the DNA endonuclease present in avian leukosis and sarcoma viruses (ASLVs) suggested its derivation from the 3′ end of *pol*[4,10], a suggestion that has since been extended to the endonucleases of other viruses (see below). The source of the protease has been more enigmatic: though associated with the carboxyterminal product of the ASLV *gag* polyprotein[4], protease activity has not been detected in murine leukemia virus (MLV) *gag* proteins [11]. Analysis of a *pol* frameshift mutant competent to process its *gag* polyposition and comparison of the amino-terminal sequence of MLV reverse transcriptase with the nucleotide sequence of *pol*[12] suggests that there is room in the region of *pol* immediately downstream of *gag* to encode the protease isolated from MLV particles.

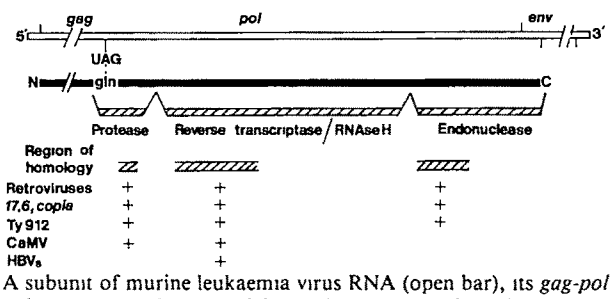
Strong confirmation of these predictions is now in hand. A partial amino acid sequence of a protease purified from MLV corresponds to the sequence predicted from the 5′ end of *pol*[13] (also see below); and a short in-frame deletion mutation, engineered in the same region, cancels processing of both *gag* and *gag-pol* precursors[14]. (The mutant appears to assemble virus particles with normal efficiency and, suprisingly, to contain normal levels of *in vitro* reverse transcriptase activity, though unable to synthesize viral DNA upon infection.)

Site-directed mutagenesis has also been used to document the importance of the putative endonuclease coding domain at the 3′ end of the *pol* genes of MLV[15,16], ASLV[17], and spleen necrosis virus (SNV)[18]. Both deletion and point mutations in this region of cloned MLV and SNV DNA produce virus particles that synthesize apparently normal linear and closed circular DNA intermediates after infection, but fail to integrate viral DNA with the efficiency and precision characteristic of wild type virus[15,16,18].

What are the biological roles of the endonuclease? At least two recombinatorial functions can be envisioned. First it may be responsible for a staggered cutting of chromosomal DNA (ostensibly in a sequence-independent manner) to yield the virus-specified four, five, or six base-pair duplications of host sequence that are found to flank proviral DNA. Second, the endonuclease may carry out sequence-specified tailoring of viral DNA so that proviruses are uniformly joined to host DNA at sites two base pairs from the ends of viral long terminal repeats (LTRs), within the inverted repeats that conclude the LTRs[19]. There are now strong reasons to believe that during infection and integration the endonuclease recognizes and is to cut circle junctions — the palindromic sequences formed when the inverted repeats at the end of unintegrated linear DNA are joined to yield circular DNA species. Experimental insertion of a small circle junction fragment into an SNV vector creates a second integration site within the viral genome[20] and integrity of the inverted repeats is mandatory for integration [21]. In addition, when an ALSV endonuclease is incubated with single-stranded viral DNA *in vitro*, it prefers to cut close to the expected sites[22].

The claim that hepatitis B viruses (BHVs) and cauliflower mosaic virus (CaMV) replicate through RNA intermediates with the help of reverse transcriptase (see ref. 23) has received striking support from computer searchers: Toh *et al.* were first to report a persuasive relatedness between the amino-terminal portion of retroviral reverse transcriptases and products of long unassigned open reading frames of HBVs and CaMV[24]. Comparisons of products of disparate retroviruses have shown that all three functional domains described for the MLV *pol* gene are strongly conserved, although not always within a single open reading frame (see below)[25-27].

Similarly successful games have now been played with the newly-determined sequences of the mobile *copia*-like genetic elements of *Drosophila* and the mobile *Ty* elements of *Saccharomyces*. These transposons had previously been recognized to resemble retroviral proviruses from their general organization and some limited sequence similarities within, and adjacent to, their LTRs (reviewed in ref. 28). Saigo, Miyata and their colleagues[27,29] have since found that protein sequences homologous to retroviral protease, reverse transcriptase, and endonuclease are encoded within the second of three long open reading frames of the *copia*-like element *17.6*, where they are arranged similarly to the domains described for MLV *pol*. In *copia* itself, which has only a single, extensive open reading frame, the endonuclease region instead lies between an upstream protease and a downstream reverse transcriptase region[30]. The yeast element, *Ty* 912, has a *copia*-like arrangement of domains within the second of two open reading frames[31].



A subunit of murine leukaemia virus RNA (open bar), its *gag-pol* polyprotein product (solid bar), the enzymes cleaved from the polyprotein (hatched bar) and regions of sequence homology between murine leukaemia virus and other genetic elements

Transposition via RNA

There is, at least, superficial congruence between the sequence relationships listed in the figure and the evidence available about their predicted functional consequences. Thus, for HBVs and CaMV, which contain reverse transcriptases but not endonucleases, the replication cycle seems to include RNA-directed DNA synthesis but not orderly or efficient integration into the host genome. Several transposable elements of *Drosophila* and *Saccharomyces* are organized in a fashion consistent with the use of a retrovirus-like integrative mechanism, dependent upon the putative endonuclease domains; but, until recently, evidence for reverse transciption has been limited to the existence of linear and circular DNA forms of *copia*-like elements that resemble unintegrated retroviral species[32-34] and virus-like particles associated with *copia* RNA that display incompletely studied reverse transcriptase activity[35].

This rather tentative situation has now been dramatically altered by experiments that show beyond reasonable doubt that *Ty* elements, like retroviral proviruses,

transpose via RNA intermediates. For what seems likely to rank among the ten best scientific stories of the year, Boeke *et al.* constructed a molecularly-tagged *Ty* element[36], in which the promoter of the 5′ LTR is replaced by the inducible *GAL1* promoter, so that *Ty* transpositions to a target yeast plasmid[37] could be shown to be dependent on transcriptional activity. Moreover, *Ty* LTRs were shown to be regenerated in each transposition cycle, in the manner of retroviral LTRs such that differences between the LTRs in the original element are eliminated from the newly synthesized copy received by the target plasmid. Finally, in a manoeuvre destined to convince even those unfamiliar with the acrobatics of retroviral replication that an RNA intermediate is used, Boeke *et al.* prove that an intron placed in an innocuous position in the moveable element can be precisely removed during transposition.

In the wake of the *Ty* results, few will question the hypothesis that *copia*-like elements of *Drosophila* and retrovirus-like elements of mammals (for example, VL-30 and intracisternal A particle DNAs[3]) also transpose and amplify their numbers through RNA intermediates, even though they lack the ability to make extracellular virus particles. But acceptance of this idea carries with it an evolutionary dilemma: are retroviral proviruses of vertebrates, with their capacity to spread as viruses from cell to cell and animal to animal, to be considered as advanced versions of more primitive elements present in all eukaryotes? Or are such elements the remnants of ancient retroviral infections, retaining only in occasional instances the capacity to mediate limited (intracellular) transposition?

Translational suppression

It has been apparent for several years that something peculiar must occur at the boundary of *gag* and *pol* (see figure) to allow synthesis of a *gag-pol* polyprotein, at 1-5 per cent the efficiency of *gag* polyprotein[3]. From the first definitions of the precursors to MLV and ASLV reverse transcriptases[38,39], at least two possibilities have been on the table: either an inefficient splicing event generates a *gag-pol* mRNA that lacks the *gag* terminator, or the terminator must occasionally be suppressed or otherwise bypassed in translation. Those favoring simple termination suppression took heart some years ago from the demonstration by Philipson *et al.*[40] that MLV virion RNA could be translated *in vitro* to yield both *gag* and *gag-pol* precursors, and that the latter was increased at the expense of the former by addition of yeast amber-suppressor tRNA. In similar experiments with Rous sarcoma virus (RSV) RNA, however, addition of suppressor tRNA provoked the synthesis of a protein only slightly longer than the *gag* precursor[41]. Nucleotide sequencing then revealed that *gag* and *pol* are in different, albeit overlapping, reading frames in the RSV genome[42], whereas MLV *gag* and *pol* are in the same frame and separated only by an amber codon[43], as anticipated from the *in vitro* translation studies.

The translational supression school thereupon fell into mild disfavour and, since all retroviruses generally solve their problems in similar ways, it was widely assumed that they must make a small amount of atypically spliced *gag-pol* mRNA, despite the absence of recognizable splicing signals. The constitutive synthesis of *gag-pol* protein *in vitro* was ascribed to inadvertent packaging of *gag-pol* mRNA.

Oroszlan and his colleagues[13] have now turned all eyes back to translational suppression, by showing unambiguously that the amber terminator of the MLV *gag* is suppressed during synthesis of the *gag-pol* polyprotein. Amino-terminal sequencing of the protease of MLV shows it to be a cleavage product of the region of the polyprotein encoded by the *gag-pol* junction. More importantly, the first four amino acids are encoded by the *gag* sequence preceding the amber codon (UAG in figure); those beginning with number six are encoded by the *pol* sequence following the terminator; and the fifth amino acid is glutamine (gln in figure) in at least 80-90 per cent of the protease molecules. What determines that the amber codon is misread as glutamine instead of termination 1-5 per cent of the time? What species of tRNA is involved? Can other genes be affected in this way?

These self-evident questions are accompanied by less obvious, but no less provocative, ones. What should we now make of RSV, in which *gag* and *pol* are out of frame? Is there a mechanism for translational frameshifting that works as regularly upon RSV RNA as amber suppression does upon MLV RNA? Oblique suggestions that this is the case have come from recent studies of yeast *Ty* elements. The two major open reading frames of *Ty* 912 are overlapping and the second has no initiation codon until well into the open frame; rigorous attempts to discover an appropriately spliced mRNA have failed; and the size of *Ty-lacZ* fusion proteins indicates that translation of the second reading frame initiates from the 5′ end of the first[31]. From this and a related study by Mellor *et al.*[44], it seems likely that translational frameshifting is required for expression of the second frame, which is encodes proteins related to the products of retroviral *pol* genes.

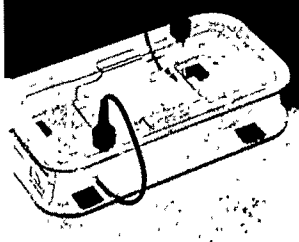
Cursory examination of the relationships between the first (*gag*-like) and second (*pol*-like) coding domains of a number of retrovirus-related elements shows a tantalizing potential for diversity in the synthesis of *pol* proteins and their homologues. In *Ty* 912, the second frame is '+1' with respect to the first[31], whereas the RSV *pol* frame is '−1' with respect to *gag*[42], *and the same is true for the Drosophila* element *17.6*[29]. On the other hand, *copia* seems to be similar to MLV, with *gag*- and *pol*-like sequences in the same frame, but not separated by a termination codon[30]. The frequency of termination codons between the *gag* and reverse transcriptase coding regions of HTLV-I[45] and II[46] and bovine leukaemia virus[47,48] demands either splicing or multiple suppression events for expression of *pol*; however, translation of sequences that begin near the 3′ end of *gag* in the '−1' frame would yield proteins related to retroviral proteases[27,49].

In sum, an astonishing amount has been learned recently about functional and structural similarities among the *pol* genes of retroviruses and related elements — a good augery for the next fifteen years of reverse transcription. □

1 Temin, H M & Mizutani, S *Nature* **226**, 1211 (1970)
2. Baltimore, D *Nature* **226**, 1209 (1970)
3 Weiss, R , Teich, N , Varmus, H E & Coffin, J (eds) *Molecular Biology of Tumour Viruses. RNA Tumor Viruses* (Cold Spring Harbor, New York, 1982)
4 Grandgenett, D P , Vora, A C & Schiff, R.D *Virology* **89**, 119 (1978)
5. Kopchick, J.J *et al J Virol* **37**, 274 (1981)
6 Nissen-Meyer, J & Ness, I *Nucl Acids Res* **8**, 5043 (1980)
7 von der Helm, K *Proc. natn Acad Sci U S A* **74**, 911 (1977)
8 Dittmar, K.J & Moelling, K *J Virol* **28**, 106 (1978)
9 Yoshinaka, Y & Luftig, R B *Cell* **12**, 709 (1977)
10 Grandgenett, D.P , Kraus, R J & Hippenmeyer, P J *Virology* **130**, 257 (1983)
11 Levin, J.G. *et al J Virol* **51**, 470 (1984)
12 Copeland, T D *et al Virology* (in the press)
13. Yoshinaka, Y , Katoh, I , Copeland, T D & Oroszlan, S *Proc natn. Acad Sci U S A* , **82**, 1618 (1985)
14 Crawford, S. & Goff, S *J Virol.* **53**, 899 (1985)
15 Schwartzberg, D *et al. Cell* **37**, 1043 (1984)
16 Donehower, L.A & Varmus, H.E *Proc natn. Acad Sci U S A* **81**, 6461 (1984).
17 Hippenmeyer, P & Grandgenett, D *Virology* **137**, 358 (1984)
18. Panganiban, A T & Temin, H M. *Proc natn Acad Sci U S.A* **81**, 7885 (1984)
19 Varmus, H E. *Science* **216**, 812 (1982)
20 Panganiban, A T & Temin, H M *Nature* **306**, 155 (1983)
21 Panganiban, A.T & Temin, H M. *Cell* **36**, 673 (1984)
22 Duyk, G , Leiss, J., Longiaru, M & Skalka, A M *Proc natn Acad. Sci U S A* **80**, 6745 (1983)
23 Varmus, H E *Nature News and Views* **304**, 116 (1983)
24 Toh, H *et al Nature* **305**, 829 (1983).
25 Pataroa, R & Haseltine, W A *Nature* **309**, 728 (1984)
26. Chiu, I M. *et al Science* **223**, 364 (1984)
27 Toh, H. *et al. EMBO J* (in the press)
28 Varmus, H E in *Mobile Genetic Elements* (ed Shapiro, J) (Academic Press, New York, 1983).
29 Saigo, K *et al Nature* **312**, 659 (1984)
30. Mount, S & Rubin, G. *Mol cell Biol* (in the press)
31 Clare, J & Farabaugh, P *Proc natn Acad Sci U S A* (in the press)
32 Flavell, A J & Ish-Horowicz, D *Nature* **292**, 591 (1981), *Cell* **34**, 415 (1983)
33 Flavell, A J *Nature* **310**, 514 (1984)
34 Mossie, K G , Young, M W. & Varmus, H E *J mol Biol* , (in the press)
35 Shiba, T & Saigo, K *Nature* **302**, 119 (1983)
36 Boeke, J D , Garfinkel, D J , Styles, C A & Fink, G R *Cell* **40**, 491 (1985)
37 Scherer, S , Mann, C & Davis, R W *Nature* **298**, 815 (1983)
38. Jamjoom, G A , Naso, R B & Arlinghaus, R B *J Virol* **78**, 311 (1977)
39 Opperman, H , Bishop, J M , Varmus, H E & Levintow, L *Cell* **12**, 993 (1977)
40 Philipson, L , Andersson, P , Olshevsky, U , Weinberg, R , Baltimore, D & Gesteland, R *Cell* **13**, 189 (1978)
41 Weiss, S *et al Cell* **15**, 607 (1978)
42. Schwartz, D , Tizzard, R & Gilbert, W *Cell* **32**, 853 (1983)
43 Shinnick, T M , Lerner, R A & Sutcliffe, J G *Nature* **293**, 543 (1981)
44 Mellor, J *et al Nature* **313**, 293 (1985)
45 Seiki, M , Hattori, S , Hirayama, Y. & Yoshida, M *Proc natn Acad Sci. U S A* **80**, 3618 (1983)
46. Shimotohno, K *et al Proc natn Acad Sci U S A* (in the press)
47 Sagata, N *et al Proc natn Acad Sci U S A* **82**, 677 (1985)
48. Rice, N *et al Virology* (in the press)
49 Sagata, N *et al FEBS Lett* **178**, 78 (1984)

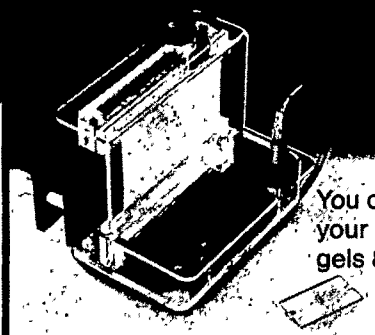
Harold E. Varmus is in the Department of Microbiology and Immunology, University of California, San Francisco, California 94143, USA.

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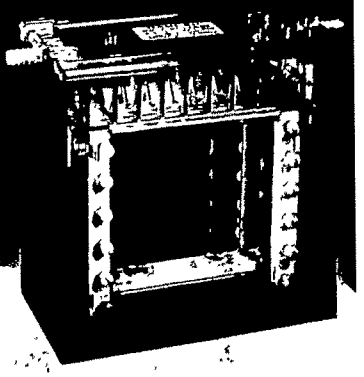
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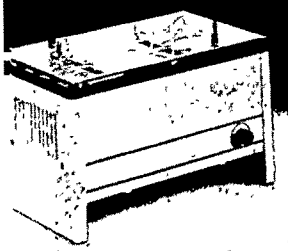
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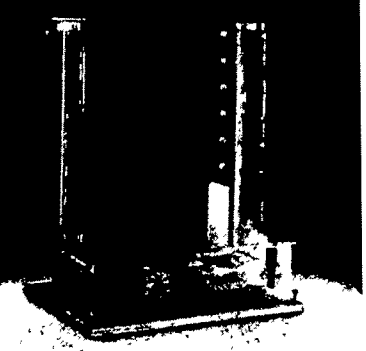
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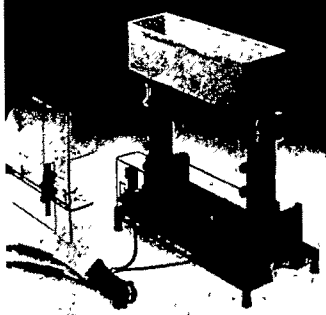
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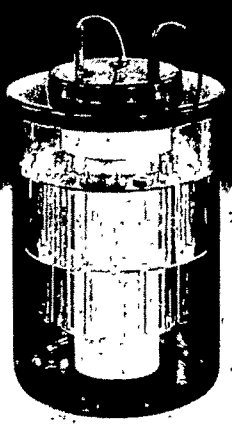
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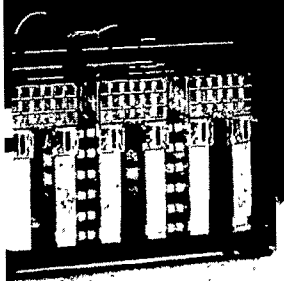
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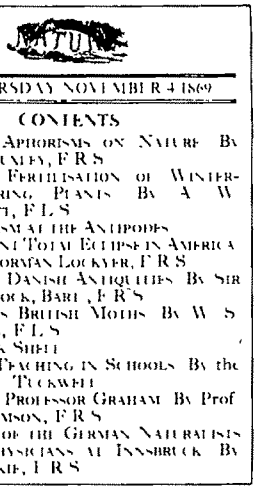
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Novel DNA sequence representations

SIR — Recent contributions from Lathe and Findlay[1] and Hayashi and Munkata[2] are manifestations of a widespread concern that the conventional (letter-sequence) representations of long DNA sequences are unsuitable for purposes other than elementary information storage. With regard to comprehensibility, such data appear to be analogous to the atomic coordinate tables seen in the specialized journals of X-ray crystallographers. Data in such form cannot be remembered, compared to other similar data, or scanned for certain features without some computer manipulations.

Lathe and Findlay's suggested sequence representation is based on four vertical lines of unequal lengths, each assigned to one of the four nucleotide bases[1]. (This idea appears to be related to the more general computer-based approach used by Daniels *et al.*[3].) For relatively short sequences the method creates attractive mnemonics capable of reflecting certain symmetry features of the sequence. However, for long DNA sequences of several thousand nucleotides this method would be limited by the same problems as those of the letter-sequence method — a compromise between excessive figure size and legibility, lack of indication of global sequence characteristics, and the impossibility of resolution adjustment. The conventional letter-sequence method seems adequate for short DNA where the conveyance of the short-range details of the information is the most important factor. Thus, the suitability of any alternative method must be tested not only on short but also on long DNA sequences.

We have proposed a graphic representation method[4-6] based on the idea of mapping the information content of a DNA sequence into a three-dimensional space curve such that the shape of the curve represents the sequence of nucleotides. A short space vector of characteristic direction is assigned to each of the four nucleotides (Fig. 1) and the curve is assembled from such vectors by joining them head-to-tail in the order of the nucleotides in the DNA sequence. The front and the side views of these 'H-curves' (or their stereoscopically viewed three-dimensional version) can provide not only short-range detail but can also reflect global nucleotide-distribution trends. For instance, DNA regions rich in certain nucleotides will result in H-curve regions shifting in characteristic directions. An important property of these curves is that they can be generated or viewed at different degrees of resolution. At high resolution the individual nucleotides of the sequence are observable but at lower resolution, (when curves are condensed to a relatively short vertical length) the regional variations of nucleotide-distribution patterns along the DNA sequence are emphasized. This latter property of our curves makes them particularly suitable for representing very long DNA sequences. (We recently published the evaluation of the H-curve of the λ genome sequence of 48,502 nucleotides[7].)

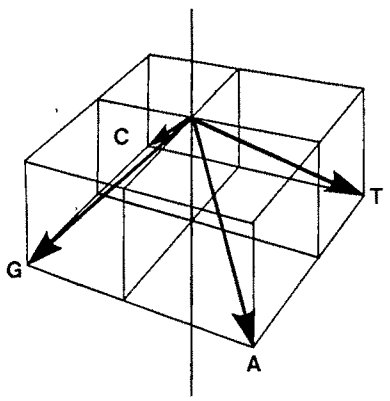


Fig. 1 Perspective illustration of the four small elemental space vectors used to depict the four nucleotides in the H-curve method of DNA sequence representation. The H curve for a DNA sequence is constructed by a head-to-tail assembly of these vectors according the nucleotide sequence of the DNA.

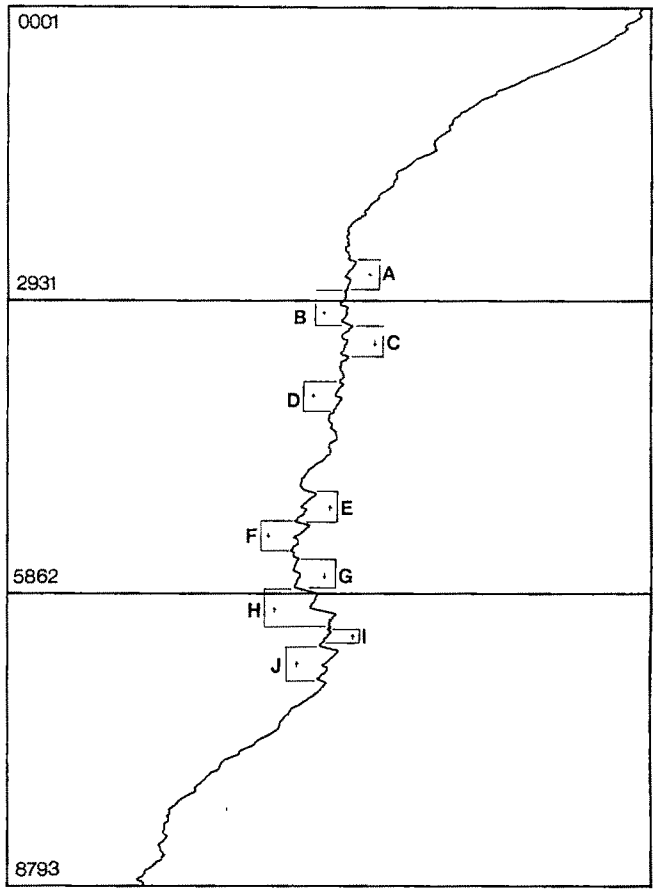


Fig. 2 The H curve of an 8,793-nucleotide-long human DNA sequence (an expressed isotype (5β) of the β-tubulin gene cluster[8]). The projection of the curve shown is the front view with the elemental vectors (here unresolvable) for the G and C nucleotides pointing to the left and those for the A and T nucleotides to the right. The vertical central portion of the curve corresponds to an unusual intron containing a cluster of ten 300-nucleotide-long *Alu* sequences (sections marked with letters). The top of the curve is the 5′ end. The vertical reference lines are 2,931 nucleotides apart and the smoothing factor[5] (*w*) was 10.

The curve shown in Fig. 2 illustrates the utility of our representation method for the display and location of characteristic repeating elements on a lengthy eukaryotic DNA sequence.

EUGENE HAMORI

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1 Lathe, R & Findlay, R *Nature* **311**, 610 (1984)
2 Hayashi, K & Munakata, N *Nature* **310**, 96 (1984)
3 Davidson, N & Szybalski, W in *The Bacteriophage Lambda* (ed Hershey, A D) 62-70 (Cold Spring Harbor Laboratory, New York, 1971)
4 Hamori, E *Fedn Proc* **40**, 1647 (1981)
5 Hamori, E & Ruskin, J *J biol Chem* **258**, 1318-1327 (1983)
6 Hamori, E *Fedn Proc* **42**, 2263 (1983)
7 Hamori, E *Gene analyt Techq* **42**, 2263 (1983)
8 Lee, M G-S, Loomis, C & Cowan, N J *Nucleic Acids Res* **12**, 5823-5836 (1984)

TATHE AND FINDLAY REPLY — Sequences of the four letters G, A, T(U) and C, are particularly intractable to visual analysis. An ideal format would (1) reveal local features such as recognition sites for enzymes interacting with nucleic acids; (2) reveal large-scale base distribution patterns, and (3) be machine-readable. It is improbable that a single method can meet all three requirements.

The methods proposed by Hamori[1] and ourselves[2] are quite different. We are nevertheless struck by the parallels between the two formats (angle-vector H-curve and line-extension) and the two methods of

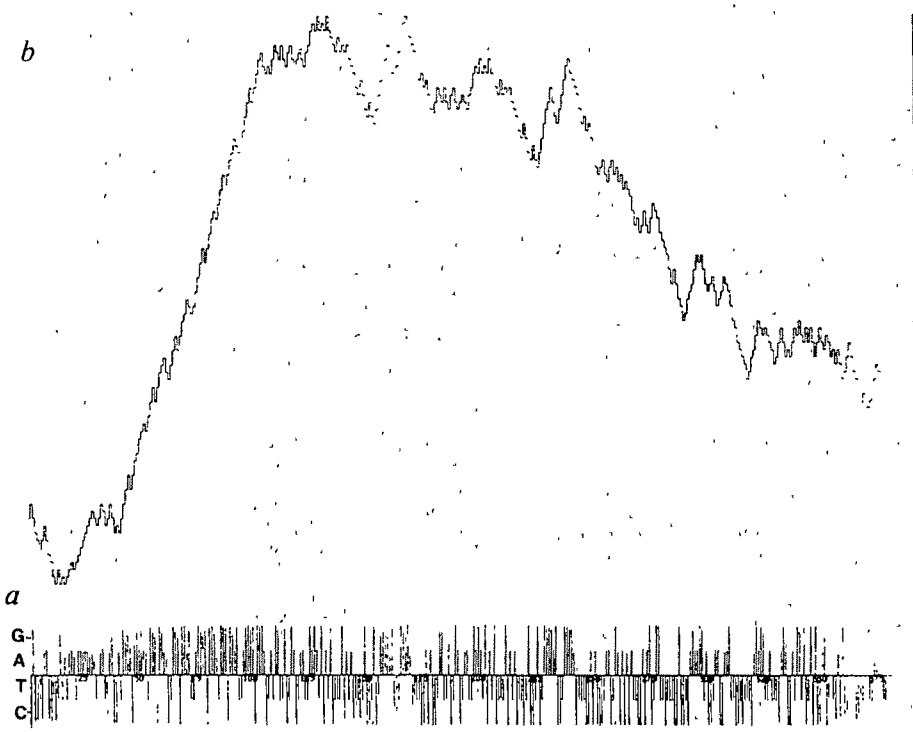


Fig. 1. The first 376 nucleotides of the SV40 genome displayed by the standard (*a*) and cumulative (*b*) line-extension formats. In both diagrams the four nucleotides G, A, T and C are given *y*-axis values of +2, +1, −1 and −2, although the scale of *b* has been reduced in this dimension. The direct 72-base pair repeat is clearly visible (region 100–250). Restriction recognition sequences form (usually) rotationally-symmetrical patterns in *a* and symmetrical peaks or troughs in *b*.

resolving oligonucleotides for nucleic acid sequencing — wandering spot[3] (Hamori) and thin-layer acrylamide gel electrophoresis[4] (our method). This superficial coincidence may reflect a limited number of possible formats.

Our first concern has been to devise a method of transferring printed sequences, using a scanning light-pen, from the printed page to an information retrieval system. Simple variants of bar-thickness identification codes were ruled out since deciphering is particularly taxing. Angle-vector methods of display such as H-curves are not inherently machine-readable although they reveal, as do plots of G/C content, features of the overall sequence profile. The base-composition curve is, to a close approximation, the first derivative of the (projected) H-curve, and as such embodies the same information. We have also examined formats which reflect the chemical structures of the four bases and which might, by comparison of non-identical recognition sequences, reveal nucleic acid–protein contact points. However, such formats were particularly difficult to interpret by eye.

We have subsequently examined a display which combines features of both the line-extension and angle-vector formats. This cumulative line extension format (which respects the extension ratios for the four bases, Fig. 1) gives similar information to that yielded by angle-vector diagrams but is less ambiguous than projected H-curves. Restriction recognition sequences appear here as symmetrical peaks. However, the format is not machine-readable and we have not proceeded further with this approach.

The two methods under discussion [1,2] meet different applications. The method we have proposed reveals local features of a DNA sequence and is limited to some 10,000 nucleotides per standard page. The display is machine-readable. In contrast, H-curves accentuate (as do G/C content plots) global features of a large-scale sequence while concealing the detail. They permit 100,000 or more nucleotides to be displayed per page. To this extent the two methods are non-overlapping and provide a visual representation of a particular nucleic acid sequence.

R. LATHE
R. FINDLAY

ARFC Animal Breeding Research Organisation,
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Edinburgh, EH9 3JQ, UK

1 Hamori, E *Gene analyt Techq* **1**, 69, (1984)
2 Lathe, R & Findlay, R *Nature* **311**, 610 (1984)
3 Sanger, F, Brownlee, G G & Barrell, B G *J molec Biol.* **13**, 373 (1965)
4 Sanger, F, Nicklen, S & Coulson, A R *Proc natn Acad Sci U S A* **74**, 5463 (1977)

Powers of ten wrongly expressed

SIR — It is surprising how often authors improperly use powers of 10, in the expression of radioactivity measurements particularly. I often read manuscripts in which the ordinates are labelled as follows: 'Radioactivity (c.p.m. × 10^{-3})' or simply 'c.p.m. × 10^{-3}' with figures ranging from 0 to 10 or so.

Since $a \times b = b \times a$; 'c.p.m. × 10^{-3}' actually means '10^{-3} c.p.m' or 'milli-counts per min'. This is obviously not what the authors mean. The proper way to label such ordinates is either '10^{-3} × radioactivity (c.p.m.)' or 'Radioactivity (c.p.m. × 10^{3})'.

A physical quantity (see page 5 in ref. 1) is the product of a pure number and a unit, that is, a particular physical quantity used as a standard, for example:

Mass = 3 × grams (or 3 g) (1)
Radioactivity = 5,000 × c.p.m. (or 5,000 c.p.m.) (2)

If one divides 5,000 by 10^3 in equation (2), then 'radioactivity' should also be divided by 10^3 (or multiplied by 10^{-3}), or 'c.p.m.' should be multiplied by 10^3.

Many authors divide both the number and the unit by 10^3, thus dividing only the right-hand side of equation (2) by 10^3. This is incorrect.

CLAUDE LIÉBECQ

European Journal of Biochemistry,
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1 International Union of Pure and Applied Chemistry *Manual of Symbols and Terminology for Physicochemical Quantities and Units 1979 Edition* (Pergamon Press, Oxford 1979)

Mass extinction times and correlations

SIR — Rampino and Stothers[1] make the interesting argument that the correlation they found[2] between ordered sequences of nine mass extinction times and nine galactic plane crossings ($r = 0.996$) is statistically significant, while the correlation that I found[3] between the same ordered extinction times and the first nine prime numbers ($r = 0.986$) is not. Of course, this leaves open the question of the significance of the correlation of the ordered extinction times and the first nine odd numbers ($r = 0.995$).

The question of how to test statistical relations between serial data is a serious and difficult one of long standing, going back at least a century to W. Stanley Jevons' attempts to show a connection between sunspots and the business cycle. I believe its resolution in the present case will probably require more data, and certainly a different analysis, than that given by Rampino and Stothers. All monotone series appear highly correlated, all regular monotone series appear even more so. This artefact aside, and even under the authors' most optimistic assumptions, the statistical evidence is simply too weak to reach any conclusion, due to the large variance (relative to the small sample size) of the times between mass extinction dates. The intriguing connection Rampino and Stothers suggest between these two phenomena may well be a real one; their analysis has not yet proved it to be real.

S. M. STIGLER

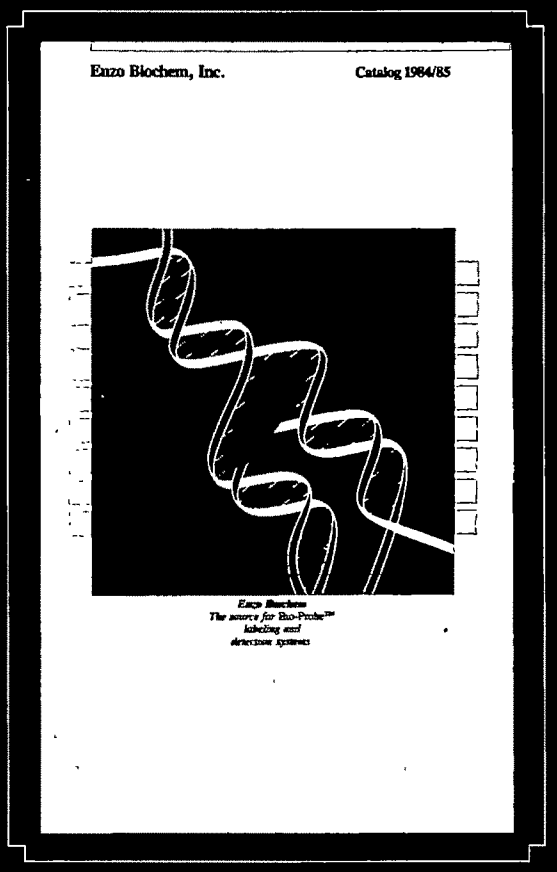
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Chicago, Illinois 60637, USA

1 Rampino, M R & Stothers, R B *Nature* **313**, 159-160 (1985)
2 Rampino, M.R & Stothers, R B *Nature* **308**, 709-712 (1984)
3 Stigler, S M *Nature* **313**, 159 (1985)

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Records of Halley's comet on Babylonian tablets

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The late Babylonian texts in the British Museum are shown to contain probable observations of Halley's comet at both its 164 BC and 87 BC apparitions. These texts have important bearing on the orbital motion of the comet in the ancient past.

HALLEY'S comet returns to the inner Solar System every 75–80 years, the precise interval depending appreciably on perturbations in its orbit induced by various planets. The comet is unique in being the only known periodic comet that regularly becomes bright enough to attract the attention of the unaided eye. As a result, no other comet has a comparable history. Since the pioneering work of Halley[1], the orbital motion has been investigated carefully and observations of every apparition as far back as 12 BC have been identified confidently[2].

Despite this impressive record, the history of Halley's comet before 12 BC is relatively obscure. At this early period, Chinese records, so detailed in later centuries, are comparatively poor, although there are two possible references in 87 BC and 240 BC[2,3]. Greek and Latin sources mention several comets occurring in ancient times, but scant attention is paid to information such as celestial location and exact date of observation. Identification of P/Halley (the prefix denotes the periodic nature of the comet) among such diverse material is very difficult, if not impossible.

A neglected source of cometary observations is the late Babylonian astronomical texts. These inscribed clay tablets were discovered about a century ago and are now mainly in the British Museum[4]. Here we present for the first time two separate Babylonian records of P/Halley from 164 BC (the only extant records of this apparition in the world) and one from 87 BC. It is hoped that these records will enable a more definite orbit of P/Halley to be traced into the ancient past and a deeper understanding of the evolution of cometary orbits to be made with respect to the recent interest in cometary catastrophism.

Orbital parameters

According to Ho Peng Yoke's[5] catalogue of oriental records of comets and guest stars, some 500 naked-eye comets have been observed over the past 2,200 years (that is, an average of one comet approximately every five years). On the basis of these statistics, there is a significant probability of another bright comet appearing in the same year as P/Halley itself; in fact several such double events are known to have occurred, most recently in 1910. Thus, it is evident that to investigate the most ancient apparitions of Halley's comet, a highly reliable orbital theory is required or an incorrect identification may well be made. Recently, four independent sets of osculating orbital elements have been derived for P/Halley by Chang[6], Yeomans and Kiang[7], Brady[8] and Landgraf[9]. All the solutions cover more than three millennia and are based on a numerical integration of the equations of motion of the comet backwards in time.

The apparent motion of a comet across the celestial sphere is only weakly dependent on small changes in orbital parameters such as eccentricity, inclination and perihelion distance. The slight differences in these parameters deduced by the various authors can safely be neglected. On the other hand, the relative motion with respect to the Earth is very sensitive to variations in the precise date of perihelion passage (T). Hence a question of prime concern is the reliability of values of T as calculated by the authors of refs 6–9.

Both telescopic and pre-telescopic measurements of the position of P/Halley enable the dates of every perihelion passage back to AD 989 to be determined accurately[2,3,7]. On only two occasions during this period do observational uncertainties exceed 0.5 day (1.5 days in AD 1222 and 0.8 days in AD 1145).

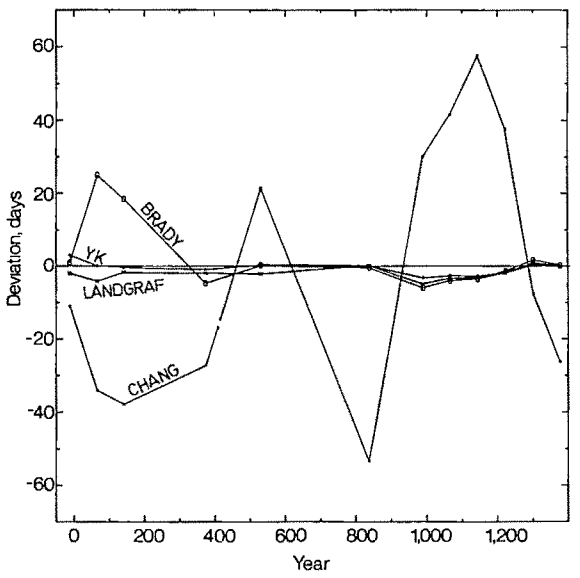


Fig. 1 Plot of the deviations between the calculated and observed dates of perihelion passage for Halley's comet between 12 BC and AD 1378 for the investigations in refs 6–9 YK, Yeomans and Kiang.

Further back in time the precision of observation is generally low but there are several notable exceptions. In AD 837, 530, 374 and 141, careful measurements of the RA of the comet by Chinese astronomers enable values of T to be deduced with uncertainties between 0.1 and 0.5 days. Finally, Chinese observations in AD 66 and 12 BC, although less accurate, still have tolerably small uncertainties of ~2 days.

Over the earlier part of this time range, the results of a comparison between observation and theory for the four investigations cited are summarized in Fig. 1. This diagram emphasizes the high accuracy of Yeomans and Kiang[7], Brady[8] and Landgraf[9] in representing observations as far back as AD 374; discrepancies in T do not exceed ~5 days at any perihelion passage since that date. Similar consistency for the three most ancient well-observed apparitions (AD 141, AD 66 and 12 BC) is obtained in refs 7, 9. However, Brady's[8] results for T are somewhat discordant in both AD 141 (~20 days error) and AD 66 (~25 days), although the accuracy is again high in 12 BC (discrepancy ~1 day). The relatively low precision of Chang's[6] solution is evident; errors of a month in T are not unusual.

The mutual discrepancies in the dates of perihelion passage as calculated in refs 6–9 back to 700 BC are shown in Fig. 2. Here, Yeomans and Kiang's data[7] have been adopted arbitrarily as the standard from which to measure deviations. From Fig. 2 it is apparent that for any apparition of P/Halley back to 87 BC the agreement between refs 7–9 is good; the maximum discord amounts to less than a month. In 164 BC and 240 BC, refs 7 and 9 still tend to support one another, discrepancies being about 15 and 40 days, respectively. However, Brady[8] deviates from these solutions by some 150 days in both cases. Beyond 240 BC, even the precise years of apparition are in dispute. The apparent lack of useful observational data before 12 BC is a serious handicap to further progress. It is hoped that our discovery

of the Babylonian records for 164 BC and 87 BC will assist in the determination of more accurate solutions in the ancient past.

Babylonian observations

The extant Babylonian cometary observations are recorded in a group of cuneiform texts called Astronomical Diaries[10]. These texts originally covered the period from ~750 BC to perhaps as late as AD 75, but only a small fraction is preserved; almost all surviving texts are now in the British Museum. The diaries contain day-by-day observations, mainly of the Moon and planets, but also of other phenomena in the sky, some of atmospheric origin[11]. Comets are mentioned in several of these diaries, the earliest known observation dating from 235 BC. Unfortunately, all contexts are broken, hence it is not possible to give a complete set of observations for the apparition of any comet.

The term used for a comet in the diaries is ṣallammu (or ṣallummu), which in other Babylonian texts can denote a phenomenon similar to a meteor or fireball[12]. No etymology of the word is known; even its reading is to some extent uncertain. A meaning 'comet' is suggested by evidence from the diaries which show the ṣallammu to remain in the sky for an extended time (several days or even weeks) so that a meteor explanation is excluded. Although a meteor interpretation cannot be excluded for some of the ṣallammu mentioned in the astrological cuneiform texts, there is no evidence to suggest anything other than a cometary identification when this term is found in the diaries.

Two apparitions of P/Halley seem to be identifiable among the records of comets in the cuneiform diaries. These can be dated confidently as 164 BC and 87 BC. For the earlier of these, two separate records are extant; although both are badly damaged, the two descriptions supplement one another. Unfortunately, only a single brief entry has survived from 87 BC, but this contains several useful details, including a single exact date.

We give below transliterations and translations of each text under the relevant year. The reference numbers preceding each transliteration give the British Museum catalogue number, relevant lines of text and, where appropriate, the location in Sachs's Late Babylonian Astronomical and Related Texts (LBAT)[4]. See page 577 for a photograph of BM 41462.

(1) 164 BC

BM 41462:16f (LBAT 380)

[...sal-la]m-mu-ú šá ina IGI-ma ina ^{d}UTU-È-A ina KASKAL šu-ut ^{d}DIŠ ina KI MÚL-MÚL u MÚL.GU$_4$-AN-NA IGI ana ŠÚ x[...]

x u ina KASKAL šu-ut ^{d}BE DIB-DIB-iq

The comet which previously had appeared in the east in the path of Anu in the area of Pleiades and Taurus, to the west [...] and passed along in the path of Ea.

BM 41628:9′ (LBAT 378)

[...š]u-ut ^{d}BE ina KI PA 1 KÙŠ ina IGI MÚL-BABBAR 3 KÙŠ ana SI NIM x[...] [...in the path] of Ea in the region of Sagittarius, 1 cubit in front of Jupiter, 3 cubits high toward the north [...]

More observations of the comet, including precise dates, may have been recorded on these same tablets, but none are now preserved. As it is part of a monthly summary, the statement in LBAT 380 that the comet 'previously had appeared in the east' suggests an observation in a preceding lunar month.

As both tablets are badly damaged, it is difficult to assess exactly how much information is missing from the original records. In both texts, the details about the comet are contained in a summary statement at the end of a month. However, neither on BM 41462 nor on BM 41628 is the month (or even the year) preserved. Both texts must be dated by astronomical calculation using the lunar and planetary observations that are also recorded.

The obverse of BM 41462, on which the comet is reported, covers much of two consecutive months. Among the observations that are useful in dating the tablet are the following: (1) During the month in which the comet "passed along in the path of Ea", Jupiter was in Sagittarius; Venus was also in Sagittarius until the middle of the month, afterwards moving into Capricornus; Mercury was in Libra. (2) On the night of the 8th(?) of this same month, in the early evening, Venus was 1 cubit below Jupiter. (The cubit was equivalent to ~2.5°; see below.) (3) On the 14th, towards morning, the Moon was 1.5 cubits in front of β Tau and 4 cubits towards the south of this star. (4) On the 23rd, towards morning, the Moon was 1 cubit in front of γ Vir and 1.5 cubits towards the south. (5) On the 28th, in the early evening, Venus was 2.5 cubits below β Cap. (6) About the 12th of the following month, Venus was 2 fingers (that is, some 0.2°) above γ Cap, whereas towards morning Mars was above α Vir.

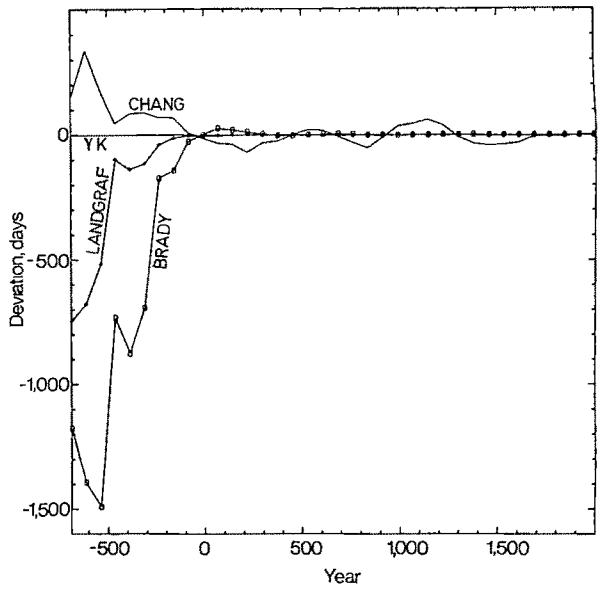


Fig. 2 Plot of the deviations between the calculated dates of perihelion passage for Halley's comet calculated in refs 6, 8, 9 relative to the results of Yeomans and Kiang[7] for the period since 700 BC. YK, Yeomans and Kiang.

On both this tablet and BM 41628, the abbreviations used for the names of stars indicate a date of writing well after 400 BC. No diaries are known later than 40 BC and we have thus limited our investigation to the period between 400 BC and the beginning of the Christian Era. During this time, the only date that satisfies the various astronomical data is the lunar month beginning on 21 October (Julian calendar) in 164 BC. From the tables of Parker and Dubberstein[13], this is equivalent to month VIII of the year 148 of the Seleucid calendar (that is, SE 148).

The tablet catalogued as BM 41628 is much more extensively damaged than BM 41462. Its obverse again covers portions of two consecutive months, but most of the entries are fragmentary. Nevertheless, several lunar and planetary observations have survived intact. These include: (1) During the month in which the comet was recorded, Saturn was in Scorpius, Mars was in Virgo and, as the cometary text states, Jupiter was in Sagittarius. (2) On the 11th of the same month. Saturn set heliacally in the end of Scorpius. (3) On the 18th, Mercury set heliacally in the east in Scorpius. (4) On the 11th of the next month, at the beginning of the night, the Moon was 1 cubit in front of α Tau and 1 cubit towards the north. (5) About the 12th of this month, Venus was 2 fingers above γ Cap. (See also entry (6) under BM 41462.)

Calculation of these observations leads to precisely the same date, the lunar month beginning on 21 October in 164 BC.

As well as dating the two texts separately, it is important to examine the direct evidence that both tablets share a common date. On each obverse the following duplicate observations are found: (1) Towards the end of the lunar month in which the comet is mentioned, the time interval between moonrise and sunrise at last visibility of the crescent Moon is given as 23,4C

uš (that is, 23.7° or ~95 min). (2) Attention has already been drawn to the observation on about the 12th of the following month when Venus was 2 fingers above γ Cap.

On the reverse of both tablets there are several identical pairs of observations, both covering two successive months: (1) On the 7th of the second month cited, both texts state that at the beginning of the night the Moon was 2/3 cubit behind β Gem. (2) On the 9th, both texts assert that at the beginning of the night the Moon was 6.5 cubits below ε Leo. (3) On the 15th, in each case the interval between sunrise and moonset is given as 5,30 uš (that is, 5.5° or 22 min). (4) As well as the above, on about the 15th of the month a lunar eclipse is reported. The two texts give few overlapping details but a separate tablet giving fuller information (BM 34037) shows that they represent portions of the same eclipse record.

The evidence is thus conclusive that the two tablets represent copies, albeit with slight variants, of the same astronomical diary. Many similar duplicate and some triplicate texts are known.

(2) 87 BC

BM 41018 rev. 8′–10′ (unpublished)

[...GE$_6$]13(?) 8 ME muš USAN ^{d}ṣal-lam-mu-ú

[...] šá ITÚ.ŠU u$_4$-mu al-la u$_4$-mu 1 KUŠ

[...] x bi-rit SI u MAR mi-ši-iḫ-šú 4 KÙŠ

On the 13th(?), the interval between moonrise and sunset was 8 degrees, measured; first part of the night, a comet [...] which in month IV day beyond day, one cubit [...] between north and west its tail 4 cubits [...]

As indicated by the brackets, the test is fragmentary. The gaps at the beginning of each line may be rather long. The passage quoted is from the 13th(?) (the number is damaged, but certainly it is at least the 12th) day of a month. Although, as before, neither the month nor the year are preserved, both may be derived uniquely from other astronomical data recorded on the same tablet. This information includes: (1) 3rd day of the same month, the Moon was 2 cubits east of Venus; (2) on about the 4th day, Mercury set in the east in Leo; (3) some time between the 4th and 12th Mars was 6 fingers above δ Cnc; (4) 13th day, a lunar eclipse which "passed by", that is, a predicted eclipse which was invisible in the longitude of Babylon; (5) some time between the 13th and 19th, Venus was 1 cubit below α Lib.

The reverse of the text mentions the capital city of Seleucia, founded in 274 BC, which sets a useful time limit. The only astronomically acceptable date is the lunar month beginning on 12 August in 87 BC, that is, month V of the year SE 225. This date is confirmed by the retrospective entry relating to month IV in the account of the comet. Calculation of the time interval between moonrise and sunset on the evening of the cometary observation (that is, 8° or 32 min) determines the date as 24 August, corresponding to the 13th of the lunar month[13]. The fact that the entry for a specific day summarizes observations going back to the previous month strongly suggests that on this day the comet had either reappeared after a period of invisibility or had been seen for the last time.

Because of the gaps in the text, one cannot be definite about certain points of syntax. Thus 'one cubit' may refer to the average daily motion of the comet or it may relate to a verb in the missing part of the next line. However, given the standard presentation of Babylonian observations in which the time reference precedes statements of position or movement, it seems probable that the present passage describes the average daily movement of the comet. The term mishu, which occasionally refers to some luminous phenomenon associated with stars, here seems to indicate the tail of the comet; passages in other texts show that it is usual to specify its direction, as indicated here.

The 'cubit', mentioned in two of the three texts, was used in Babylonian astronomy as an angular unit. It is usually estimated to be ~2° (ref. 14). From an analysis of some 20 Babylonian observations of conjunctions of the Moon with stars contained in a diary dating from SE 60 (=252 BC)[14], we have arrived at the result that 1 cubit = 2.5 ± 0.5° (s.d. per observation). The analysis also indicates that the term ina IGI ('in front of') used in the second of the 164-BC texts was a standard expression meaning 'to the west of', implying an earlier meridian transit.

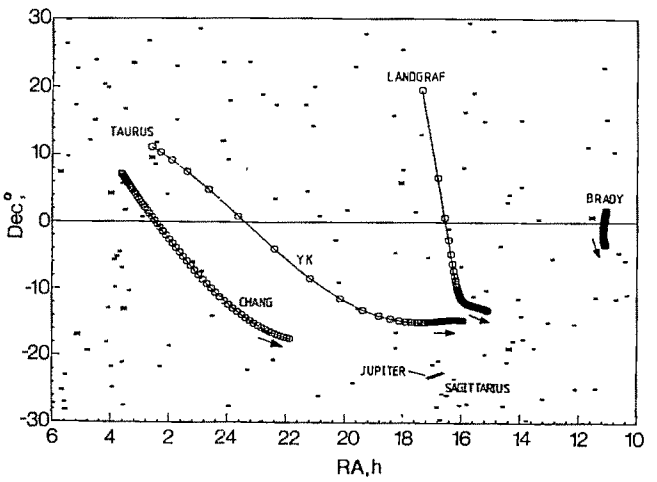


Fig. 3 The computed motion of Halley's comet in 164 BC between 22 Sep and 19 Nov for the dates of perihelion passage deduced by Brady[8] (164 BC 22 June), Landgraf[9] (164 BC 30 Oct), YK (Yeomans and Kiang)[7] (164 BC 12 Nov) and Chang[6] (163 BC 20 Jan). Positions are at daily intervals. Background stars down to mag +3.5 are shown.

The Babylonians divided the sky into three regions of declination named after gods: Enlil, Anu and Ea[15]. Of these, Anu was the equatorial region, extending between about +17° and −17°. The entire sky further north was known as Enlil, whereas that to the south was Ea. The limiting declination was based on the well known text ^{mul}APIN (possibly composed ~1000 BC), which states that the Sun spent exactly one quarter of a year in each of these regions before moving on to the adjacent one. However, it would perhaps be unwise to assume too precise a definition of Anu and Ea in analysing the motion of the comet.

Analysis of 164 BC observation

There is a brief Chinese record of a comet on a date equivalent to 6 February 162 BC (ref. 5), but this is much too late for Halley's comet. The dates of perihelion for P/Halley as computed in refs 6–9 all lie between June 164 BC and January 163 BC. The salient points of the two Babylonian accounts are as follows: (1) First appearance in the east in the Taurus region, some time before month VIII (21 October to 19 November). (2) Further appearance in the west in Sagittarius during month VIII. (3) As the difference in RA between Taurus and Sagittarius is as much as 14 h in the direct sense, it seems probable that the motion of the comet was retrograde. (4) The position relative to Jupiter (then in Sagittarius, according to calculation) was recorded at some time between 21 October and 19 November (the Babylonian month VIII). The fact that a difference in longitude (some 2.5°), as well as latitude is mentioned, indicates that this is not a record of closest approach. The reason for recording this rather careful measurement is obscure.

Figure 3 is a chart of the sky in the range of RA from 10 to 6 h and dec. from +30° to −30° at the epoch 164 BC. The region of Taurus (where the comet seems to have been first observed) and Sagittarius (where it was later observed) are marked. The range of positions computed from the JPL Ephemeris DE102 (ref. 16) for Jupiter during month VIII is indicated by crosses. The four roughly vertical tracks (composed of small squares) denote the computed motion of Halley's comet during months VII and VIII (22 September–19 November) on each of the dates of perihelion passage deduced by Brady (22 June), Landgraf (30 October), Yeomans and Kiang (12 November), all in 164 BC, and Chang[6] (20 January 163 BC). In each case the general direction of travel is from north to south, the individual squares representing positions of the comet at daily intervals. Only on the solution of Yeomans and Kiang[7] does the computed path approximate to the observational record. It is evident that merely

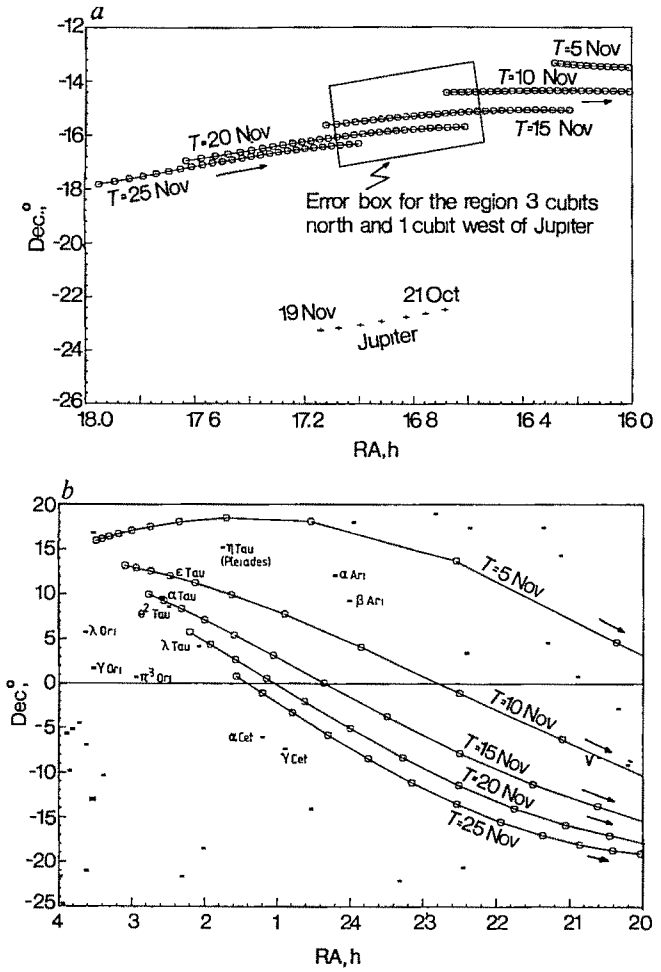


Fig. 4 *a*, Computed paths of Halley's comet in 164 BC between 21 Oct and 19 Nov (month VIII) for dates of perihelion (T) between 5 Nov and 25 Nov. The motion of Jupiter in this interval is shown and also the region of sky 3 cubits (7.5 ± 1.5°) north and 1 cubit (2.5 ± 0.5°) west of the planet as observed by the Babylonians. *b*, Computed paths of Halley's comet in 164 BC from first visibility for T between 5 Nov and 25 Nov. Stars in the Taurus region, where the comet appeared, are labelled.

varying the date of perihelion passage of Halley's comet can achieve a highly satisfactory representation of the observed motion, including the close approach to Jupiter whilst in Sagittarius. In our opinion, this is sufficient grounds for a firm identification of the recorded comet with P/Halley.

Because Halley's comet passed unusually close to the Earth (some 0.1 AU for the date of perihelion deduced by Yeomans and Kiang), small changes in T appreciably alter the relative motion of the two bodies. This circumstance is helpful in fixing narrow limits to the range of dates over which the perihelion can actually lie. We shall first consider the close approach to Jupiter during month VIII (21 October–19 November).

Figure 4*a* covers the region of sky from RA 16 to 18 h and dec. −12° to −26° at 164 BC. The computed motion of Jupiter (eastwards) during month VIII and the region 3 cubits (7.5 ± 1.5°) north and 1 cubit (2.5 ± 0.5°) west of the planet are shown. Following Babylonian practice, the relative positions of comet and planet are assumed to be measured on the ecliptic system, but there would be little difference if an equatorial frame had been used.

Computed tracks of Halley's comet moving from east to west during month VIII are shown in Fig. 4*a* for the following dates of perihelion: Nov. 5, 10, 15, 20 and 25 (each at 0 h ET). To agree with observation, some part of the computed path of the comet should intersect the error box. The indicated range of T is thus between 9 and 26 November. If T is altered by one day, the resultant east–west displacement of P/Halley is some 1.2° so that any observational error in the relative position of the comet and Jupiter can probably be neglected. It seems probable that the original tablet would record the precise date of conjunction with Jupiter and it is regrettable that because of the loss of this information only a range of T covering 17 days can be deduced. Nevertheless, this is much smaller than the scatter among the various dates derived from numerical integration.

As mentioned earlier, Jupiter was only a little to the east of the Sun during month VIII and the visibility of the comet needs to be considered. Based on our investigation of Chinese observations of the heliacal setting of P/Halley (see below under the analysis of the 87 BC record) we conclude that the comet should have been visible to the unaided eye whilst near the marked region. Hence it is not possible to restrict further the range of T on the grounds of limited visibility.

The observation made "in the east ... in the area of Pleiades and Taurus" is less accurate but gives an independent result. The precise date is no longer extant but it must be earlier than month VIII. Far-eastern records indicate that Halley's comet rarely remains visible to the unaided eye for >50 days so that first sighting is unlikely to have been earlier than month VI. During months VI and VII, Taurus would be high in the eastern sky at about midnight and the comet would thus be well placed for viewing in a dark sky. Figure 4*b* is a chart of the sky in the range RA 20 to 4 h and dec. −25° to +20°. For each of the following dates of perihelion: 5, 10, 15, 20 and 25 November, the computed motion of Halley's comet is shown. The individual tracks begin with the location of the comet at what we infer to be its earliest probable date of discovery. We have investigated the discovery magnitude of P/Halley throughout much of its history using some 20 dates of first sighting reported in various far-eastern records. From the empirical formula of Morris and Green[17], based on the observations in 1910, we find that the comet is seldom detected with the unaided eye even in a dark sky until it becomes fairly bright, between about +3.5 and +4 mag. (There is only a single instance of discovery when the calculated magnitude was fainter than +4, that is, +4.3 mag in 12 BC.) This is, of course, well above the threshold brightness for the eye (around +6 mag) but these early observers, unlike their modern counterparts, had no advance warning. We have thus assumed a discovery magnitude of +4 at Babylon in 164 BC.

Before attempting to refine the range of T already deduced, the identification of MÚL-MÚL (rendered above as Pleiades) and MÚL.GU$_4$-AN-NA (Taurus) must be considered. The modern equivalents of the various Babylonian star groups have been investigated in detail by Kugler[18], Schaumberger[15], Gössmann[19] and, more recently, Reiner[20]. From these works it is apparent that MÚL-MÚL ('The Stars') was identical to the Pleiades group whereas MÚL.GU$_4$-AN-NA ('The Bull of Anu') was centred on α Tau and the Hyades.

From the close approach to Jupiter, a date of perihelion passage for Halley's comet between 9 and 26 November has already been derived. Referring to Fig. 4*b*, it is not possible to use the Pleiades/Taurus observation to refine the earlier of these two limits, as for any T between about 7 and 16 November P/Halley would pass directly between η Tau and α Tau/Hyades. However, for dates of perihelion after about 24 November, the comet would probably not be noticed until it had moved into Cetus (MÚL-IKU). Hence we can now place firm limits on the date of perihelion for Halley's comet in 164 BC between 9 and 26 November, the latter limit possibly being as early as 24 November.

Analysis of 87 BC observation

Apart from the Babylonian text, there is a brief Chinese report of a comet appearing during this year which may relate to P/Halley[2,3]. However, only the month and general area of sky in which the comet appeared are given and we shall not consider this observation further. It is unfortunate that the cuneiform record is also brief. Despite this, several important deductions can be made. These may be summarized as follows: (1) As suggested above, there are good reasons for believing that the

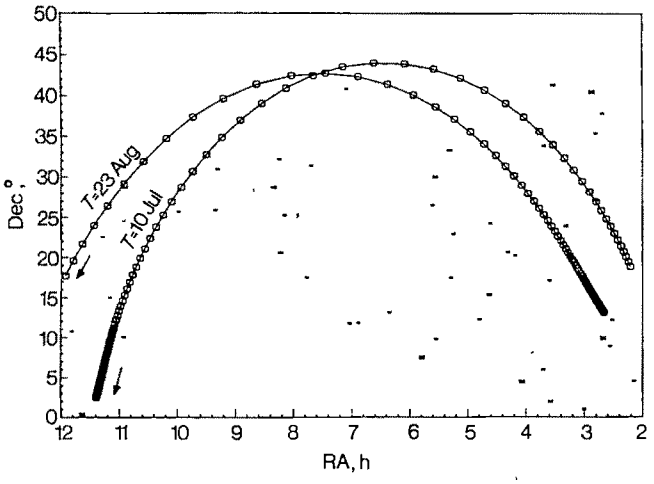


Fig. 5 The computed motion of Halley's comet in 87 BC for both the lower and upper limits of the theoretical T, that is 10 Jul (ref. 8) and 23 Aug (ref. 9) over the time range 13 Jun to 9 Sep (months III–V).

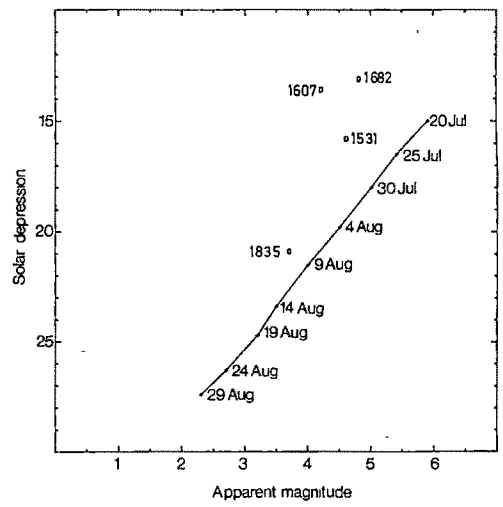


Fig. 6 Plot of the solar depression as a function of the calculated apparent magnitude of P/Halley on the evening of 24 Aug in 87 BC for dates of perihelion between 20 Jul and 29 Aug with, for comparison, the circumstances at the last recorded visibility (in Korea) in AD 1531, 1607, 1682, 1835.

observation reported on 24 August represented either reappearance or last visibility. Such a circumstance is unlikely to result from unfavourable weather, for during the summer months the skies are almost cloudless in this part of the world. (2) The comet was seen 'day beyond day' (u_4-mu al-la u_4-mu) during the 4th lunar month (14 July–11 August), which seems to imply a lengthy period of visibility during this month. (3) We add that the probability of our text referring to P/Halley, rather than some other comet, is high. The various computed dates of perihelion passage lie in the range 10 July–23 August. Calculation shows that on any of these solutions, Halley's comet would be well placed for northern observers during the summer of 87 BC (Fig. 5) and fairly bright, approaching 0 mag according to the formula of Morris and Green[17].

Figure 5 shows the computed motion of Halley's comet in a general easterly direction during months III, IV and V (13 June–9 September) in 87 BC on the dates of perihelion computed by Brady[8] (10 July) and Landgraf[9] (23 August). These represent the extreme calculated dates; Yeomans and Kiang[7] deduced 6 August, whereas Chang[6] gave 15 August. For any of these values of T, the comet never approached very close to the Earth (minimum distance ~0.45 AU) so that the motion was unusually regular. The calculated daily motion, indicated by small squares on the diagram, was always $>1°$ and $<6°$ for at least a month. As both of these values are within about a factor of two of 1 cubit, this seems to explain the reference to this quantity in the text, that is, 'day beyond day one cubit'.

As the displacement in the position of P/Halley in response to a change in T was fairly linear, it is possible to summarize the main characteristics of the apparent motion almost independently of the precise date of perihelion. On any of the four solutions cited, the comet would first reach the estimated limiting discovery brightness (+4 mag) while some 45° west of the Sun. It would then be fairly high in the eastern sky before dawn. Moving quickly eastwards, the comet would pass conjunction with the Sun approximately 10–15 days later, now becoming an evening object. Gradually diminishing in speed, it would reach a maximum elongation of only ~35° east of the Sun after a further 15 days or so. Some time later the comet, approaching a second conjunction with the Sun and growing progressively fainter, would probably set heliacally, being lost to view in the evening twilight.

From the above outline, it is apparent that the Babylonian record indicates observations both before and after the first conjunction with the Sun. The tail could only have pointed north-west before this conjunction. However, on 24 August, the date of the preserved diary entry, the comet was said to be visible in the "first part of the night" and thus to the east of the Sun. (The Babylonian astronomers divided the interval between sunset and sunrise into three roughly equal parts.) On the basis of brightness considerations, it can be deduced that by 24 August Halley's comet was probably well past conjunction with the Sun. For the comet to have been just emerging after conjunction would require an estimated discovery date (+4 mag) no earlier than about 13 August. As month IV ended on 11 August and the computed magnitude was changing by about 0.2 daily, there would seem to be no possibility of observation 'day beyond day' during this month under these circumstances. Thus, we infer that the most probable interpretation of the observation on 24 August is final visibility.

Before analysing the 24 August observation, we note that it is possible to fix useful limits on the date of perihelion in 87 BC from the additional information supplied in the Babylonian record. For T before about 20 July, the tail of Halley's comet could only have pointed north-west earlier than month IV, but such an early date does not seem to be implied in the text. If T was after about 30 August, the period of visibility in month IV would probably be too short to be described as 'day beyond day'. These limits may be narrowed further on the assumption of last sighting on 24 August.

Figure 6 illustrates the circumstances relating to the visibility of P/Halley on the evening of 24 August in 87 BC for dates of perihelion between 20 July and 29 August. The abscissa gives the apparent magnitude of the comet as deduced from the empirical formula of Morris and Green[17]. The ordinate is the depression of the Sun below the western horizon at the moment when the nucleus of the comet set (refraction being ignored). For the earlier dates of perihelion, the comet would set in strong twilight. The best time for observation would then be roughly midway between sunset and the setting of the comet itself, when the effects of the twilight glow and atmospheric absorption were optimized (although the tail would then be pointing in a roughly vertical direction, it would be very short on account of the comet's considerable distance from the Earth, some 1.5 AU for T before about 1 August).

The precise date of heliacal setting for a celestial body depends on various factors (largely of atmospheric origin) and is therefore best treated empirically. To provide some parallel data, we have analysed several dates of the last sighting of P/Halley as reported in far Eastern history. Such information is fairly regularly available over the past two millennia but before ~AD 1400 only summary records are now extant. Thus, there is a distinct possibility of copying errors, but for later centuries detailed chronicles are available from both China and Korea. The Korean works are particularly useful for our purpose because they contain day-to-day reports of astronomical phenomena, usually

stating when cloud prevented observation. Calculations based on the accurately known dates of perihelion at this relatively late period enable several instances of heliacal setting to be identified. The dates of these are 1835 November 17, 1682 October 7, 1607 November 7 and 1531 September 15. In each case the computed magnitude and solar depression are plotted in Fig. 6 (represented by circles).

Reference to Fig. 6 shows that there is some scatter among the Korean data, the 1835 solution lying somewhat below the others. The most probable explanation involves variations in the intrinsic brightness of Halley's comet from one apparition to the next, although the oriental records of first visibility show no significant long-term trends[21]. It is not possible to make any accurate deductions regarding perihelion in 87 BC, but the following remarks seem relevant: (1) Unless Halley's comet was unusually bright in 87 BC, the date of perihelion is unlikely to have been before about 25 July. For earlier dates the computed magnitude on 24 August was close to the normal limit for the unaided eye while atmospheric absorption (some 1.5 mag at optimum visibility) and the twilight glow would probably reduce the brightness well below the visual threshold. (2) For dates of perihelion after about 15 August both the calculated apparent magnitude and solar depression were very favourable and there should have been no difficulty in observing the comet on 24 August.

In summary, we infer a likely date of perihelion in 87 BC between about 25 July and 15 August. If we have misinterpreted the nature of the observation on 24 August, the latter limit would, of course, be invalid and perihelion might then lie anywhere between 25 July (to render the comet visible at all on the recorded date) and the end of August. However, the mere fact that in a day-to-day diary covering (originally) several months the Babylonian astronomers felt it sufficiently importa to provide a detailed retrospective entry on a specific day (2 August) seems consistent with an abrupt change in the visibili of the comet at that time; this we have presumed to be heliac setting.

Conclusions

We believe that the evidence that Halley's comet was observ in Babylon at its apparitions in both 164 and 87 BC is convincin The records, although badly damaged, enable fairly narro limits to be placed on the dates of perihelion in both yea between about 9 and 24 November in 164 BC and between abo 25 July and 15 August in 87 BC. Of the four numerical integr tions investigated here, only that of Yeomans and Kiang[7] pr vides acceptable dates of perihelion in both years. This presur ably results from the incorporation in their solution of sever rather accurate ancient and medieval observations, rather th using a purely theoretical analysis. In particular, their identific tion of a brief Chinese record in 240 BC as the most ancie reliable sighting of the comet finds support here. The Babyloni observations may prove useful in the integration of the moti of P/Halley further backwards in time, possibly leading to t recognition of still earlier records of this most famous of comets.

We thank Mr C. B. F. Walker of the Department of Weste Asiatic Antiquities, British Museum and Professor A. Wolfendale FRS, Department of Physics, University of Durha for reading this paper and offering several valuable suggestion The original deciphering and identification of the Babyloni texts investigated here was the work of the late Professor A. Sachs, Brown University, USA and this paper is dedicated his memory.

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1 Halley, E *Phil. Trans R. Soc.* **24**, 1882 (1705).
2 Kiang, T *Mem R. astr Soc.* **76**, 27-66 (1972)
3 Stephenson, F R & Yau, K K C *J Brit Interplanet Soc.* (in the press)
4 Sachs, A J *Late Babylonian Astronomical and Related Texts* (Brown University Press, Providence, 1955)
5 Ho Peng Yoke *Vistas Astr* **5**, 127-225 (1962)
6 Chang, Y C *Chin astr* **3**, 120-131 (1979)
7 Yeomans, D K & Kiang, T *Mon Not R. astr. Soc.* **197**, 633-646 (1981)
8 Brady, J L *J Brit astr. Assoc.* **92**, 209-215 (1982)
9 Landgraf, W *Astr Astrophys* (in the press)
10 Sachs, A J *J Cuneiform Studies* **2**, 271-290 (1948)
11 Sachs, A J *Phil Trans R. Soc.* **A276**, 43-50 (1974)
12 *The Chicago Assyrian Dictionary* Vol 16, 74-75 (Chicago, 1962)
13 Parker, R. A & Dubberstein, W H *Babylonian Chronology. 626 BC to AD 75* (Bro University Press, Providence, 1956)
14 Aaboe, A *Centaurus* **24**, 14-35 (1980)
15 Schaumberger, J *Sternkunde und Sterndienst in Babel—Erganzungsheft* 3 (Aschendorffsc Munster, 1935)
16 Newhall, X X, Williams, J G & Standish, E M *Astr Astrophys* **125**, 150-167 (1983)
17 Morris, C S & Green, D W E *Astr J* **87**, 918-923 (1982)
18 Kugler, F X *Sternkunde und Sterndienst in Babel* (Aschendorffsche, Munster, 1907-192
19 Gossmann, F. in *Sumerisches Lexikon (IV, 2)* (ed Deimel, A) (Pontifical Biblical Institu Rome, 1950)
20 Reiner, E *Babylonian Planetary Omens*, in G Buccellati (ed), *Bibliotheca Mesopotam* (II, 2), Malibu (1981)
21 Stephenson, F R & Yau, K K C *New Scient* **103**, 30-32 (1984)

Six unidentified reading frames of human mitochondrial DNA encode components of the respiratory-chain NADH dehydrogenase

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The products of six unidentified reading frames of human mitochondrial DNA are precipitated from a mitochondrial lysa by antibodies against highly purified native beef heart NADH-ubiquinone oxidoreductase (complex I). These produc are enriched greatly in a human submitochondrial fraction enriched in NADH-Q_1 and NADH-$K_3Fe(CN)_6$ oxidoreducta activities. We conclude that the six reading frames encode components of the respiratory-chain NADH dehydrogenase.

ONE of the most remarkable findings from the sequence analysis of mitochondrial DNAs from several mammalian sources[1-4], *Xenopus laevis* (B. Roe, personal communication) and *Drosophila* species[5-7] has been the discovery that more than ha of the informational content of animal mtDNA resides in eig unidentified reading frames (URFs). These genes, with o

exception, have no obvious amino-acid sequence homology to any yeast mtDNA reading frames. The demonstration that URFs with partial homology to several of the mammalian mtDNA URFs occur in mtDNA from other lower eukaryotic organisms, as first observed in *Aspergillus nidulans*[8,9] and then in *Neurospora crassa*[9] and protozoa[10,11], strongly supported the idea that these URFs code for functional proteins. Direct evidence that the URFs are functional genes has been provided by the identification of translation products corresponding to most of the human URFs, achieved by the use of antibodies directed against peptides predicted from the DNA sequence. This approach has led to the identification of the polypeptides encoded in URFA6L (ref. 12), URF1 (ref. 13), URF3 (ref. 13), URF2, URF4 and URF4L (P.M., A.C., M. Riley, B. Cottrell, R.F.D. and G.A., in preparation).

Little has been discovered about the physiological role of mtDNA URF products from animal and other species. The product of the smallest URF, URFA6L, has a low but significant amino-acid sequence homology and other structural similarities[14,15] to the product of a newly identified yeast mtDNA gene (*aap1*)[14], which seems to be a component (subunit 8) essential for the assembly of a functional H^+-ATPase complex; these observations have suggested that the URFA6L product is associated with the mitochondrial H^+-ATPase[14,15]. The other URF products are hydrophobic proteins[16] and, therefore, are presumably associated with the inner mitochondrial membrane. Among the enzyme complexes of this membrane, the NADH–ubiquinone oxidoreductase (complex I, ref. 17) and the succinate–ubiquinone oxidoreductase (complex II, ref. 18) seemed to be good candidates for containing some of the URF products, despite the previous failure to detect mitochondrially synthesized polypeptides in NADH–ubiquinone oxidoreductase of rat source[19]. The NADH–ubiquinone oxidoreductase (hereafter referred to as respiratory-chain NADH dehydrogenase or complex I) was of particular interest because it consists of a shell of $\geqslant 15$ hydrophobic polypeptides surrounding a core containing the low-relative molecular mass (M_r) NADH dehydrogenase or flavoprotein fragment and the iron–protein fragment[20,21]. Here we describe immunoprecipitation experiments using antibodies raised against purified beef heart complex I or against synthetic peptides predicted from the human URF sequences and enzyme fractionation studies; the results of the three approaches strongly suggest that the products of six of the human URFs, URF1, URF2, URF3, URF4, URF4L and URF5, are components of the respiratory-chain NADH dehydrogenase.

Products of mitochondrial genes

Figure 1 shows the present status of the polypeptide assignment to the reading frames of human mtDNA. Most of these assignments have been made by the use of anti-peptide antibodies; cytochrome *c* oxidase subunits I, II and III (COI, COII and COIII) and cytochrome *b* have been identified by analysis of the site of synthesis and electrophoretic properties of the subunits of purified human cytochrome *c* oxidase complex[22] and, respectively, purified human cytochrome b–c_1 complex (C.-J. Doersen and G.A., in preparation). The mitochondrially synthesized component 1 ($M_r \sim 51{,}000$; ref. 23) has been identified tentatively as the product of URF5 (expected $M_r = 66{,}600$; ref. 1), because this is the only gene that could code for such a large polypeptide. The discrepancy between the expected and the estimated size can be explained by the well-known bias in size estimates based on SDS-polyacrylamide gel electrophoresis of mtDNA-encoded hydrophobic proteins[12,13]. Thus, it seems that the main translation products of the human mitochondrial genes have been identified.

Reaction with anti-peptide antibodies

To determine whether the URF products are part of a protein complex, several individual URF-specific anti-peptide antisera were tested on a Triton X-100 HeLa cell mitochondrial lysate; it seemed possible that at least some of the antigenic peptides in such a complex would be accessible to their respective antibodies. Indeed, we found that each antiserum tested had a tendency to precipitate other URF products as well as the polypeptide against which it had been raised. This was seen most clearly with an antiserum against the carboxy-terminal heptapeptide of the URF4L product (anti-URF4L-C). This antiserum precipitated only one component, component 26, from an SDS mitochondrial lysate (Fig. 2*a*), which represents the URF product against which it had been prepared (P.M. *et al.*, in preparation). In striking contrast, the same antiserum precipitated components 1 (URF5), 4 and/or 5 (URF4), 11 (URF2), 12 (URF1) and 24 (URF3) as well as component 26 from Triton X-100 mitochondrial lysate (Fig. 2*b*). The same phenomenon was observed with an antiserum against the carboxy-terminal nonapeptide of the URF4 product (anti-URF4-C) (Fig. 2*a*, *b*) and with anti-URF2-N and anti-URF3-C antisera (not shown); each of these antisera precipitated its respective polypeptide, as well as a small amount of the other five URF products of the group specified above.

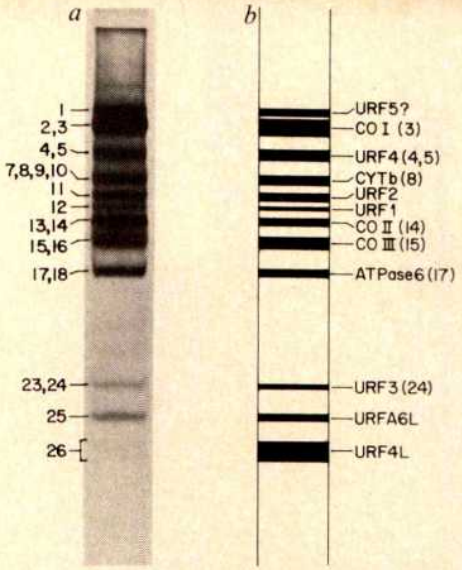


Fig. 1 Polypeptide assignment to the human mitochondrial genes. *a*, Electrophoretic pattern in an SDS/urea-polyacrylamide gel of the HeLa cell mitochondrial translation products labelled *in vivo* with ^{35}S-methionine in the presence of 100 μg ml^{-1} emetine and numbered according to Ching and Attardi[23]. *b*, Pattern of the polypeptides assigned to individual reading frames of human mtDNA. Anti-peptide antibodies were used for the polypeptide assignment to URF1, URF3, URFA6L, ATPase 6 (refs 12, 13), URF2, URF4 and URF4L (P.M. *et al.*, in preparation); the polypeptides corresponding to COI, COII and COIII were identified by analysis of the subunits of purified human cytochrome *c* oxidase[22], and the product of the cytochrome *b* gene by analysis of the subunits of purified cytochrome b–c_1 complex (C.-J. Doersen and G.A., in preparation). Numbers in parentheses indicate the polypeptide within each multicomponent band that corresponds to the indicated reading frame.

All immunoprecipitates produced from a Triton X-100 lysate by anti-URF4, URF4L, URF2 and URF3 antibodies exhibited a variable, generally relatively small amount of cytochrome *c* oxidase subunits I, II and III, but we did not detect cytochrome *b* or ATPase subunits 6 and 8 (URFA6L product). It seems probable that the presence of cytochrome *c* oxidase subunits in the specific precipitates reflects an incomplete dissociation or a secondary aggregation of these subunits in the experimental conditions used here. Even so, the tendency of the products of URFs 1, 2, 3, 4, 4L and 5 to be co-precipitated by the individual anti-peptide antibodies suggested that these components are associated with each other in some kind of complex.

Reaction with anti-complex I antibodies

To test the possibility that the putative complex containing URF1, URF2, URF3, URF4, URF4L and URF5 products is the respiratory-chain NADH dehydrogenase (complex I), we used

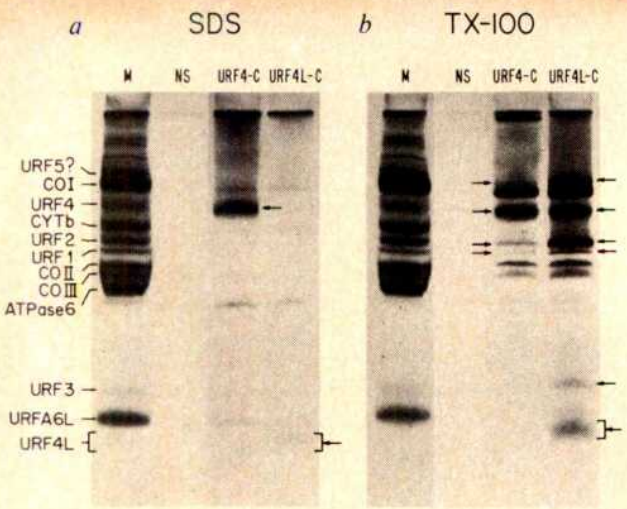


Fig. 2 Co-precipitation of several URF products by individual URF-specific antipeptide antisera. Shown are fluorograms, after electrophoresis through SDS/urea-polyacrylamide gels, of HeLa cell mitochondrial translation products immunoprecipitated by antibodies against peptides corresponding to specific regions of URF4 and URF4L. γ-Globulins from antisera against the carboxy-terminal nonapeptide of the URF4 product (URF4-C) or the carboxy-terminal heptapeptide of the URF4L product (URF4L-C) or from normal serum (NS) were incubated with an SDS lysate (*a*) or a 0.5% Triton X-100 (TX-100) lysate (*b*) of ^{35}S-methionine-labelled mitochondria. M, Pattern of mitochondrial translation products.

Methods. Antisera were prepared as described elsewhere (ref. 12 and P.M. *et al.*, in preparation). A γ-globulin fraction, free of anti-bovine serum albumin antibodies[12], was used in these experiments. For the selective labelling of the mitochondrial translation products, HeLa cells grown for 22 h in the presence of 40 μg ml^{-1} chloramphenicol[27] were labelled with ^{35}S-methionine (1,040 Ci mmol^{-1}; 19 μCi ml^{-1}) for 2 h in the presence of 100 μg ml^{-1} cycloheximide in methionine-free medium supplemented with 5% dialysed calf serum, then pelleted, washed once in complete medium, resuspended in the same medium at 10^5 cells per ml, and incubated for an additional 18 h. The mitochondrial fraction was prepared as described previously[12]. *a*, An SDS lysate of the mitochondrial fraction was prepared and aliquots (2×10^5 c.p.m., 220 μg) were immunoprecipitated at room temperature with 250 μg of γ-globulins from each of the antisera or from NS by a modification of the previously described procedure[12], to be described in detail elsewhere (P.M. *et al.*, in preparation). The immunoprecipitated products were run on an SDS/urea-polyacrylamide gel[12]. *b*, For immunoprecipitation of intact complexes of the inner mitochondrial membrane, all operations were carried out at 4 °C. Samples of mitochondrial suspension (2×10^5 c.p.m., 220 μg) were resuspended in 50 μl 0.005 M Tris-HCl, *p*H 6.7 (25 °C), 0.002 M methionine, 0.001 M phenylmethylsulphonyl fluoride, 0.5% (w/v) Triton X-100, at a concentration of 5 mg protein ml^{-1} and incubated for 20 min. After diluting the samples fourfold with the same solution containing 1 mg ml^{-1} ovalbumin (incubation buffer), methanol was added to 2% and the mixtures incubated for a further 10 min. After addition to each sample of 125 μl formaldehyde-fixed *Staphylococcus aureus*, Cowan strain I suspension (Zysorbin, Zymed), prewashed with and suspended in incubation buffer, the mixtures were incubated for 30 min and then centrifuged in an Eppendorf microfuge for 2 min. To each supernatant, 70 μl incubation buffer and 250 μg γ-globulin fraction in 70 μl phosphate-buffered saline were added and the mixtures incubated for 1 h. After addition of a 10-fold protein excess of a 0.5% Triton X-100 mitochondrial lysate (12.5 mg ml^{-1}) from unlabelled cells and SDS to 0.1%, the samples were mixed with 125 μl Zysorbin suspension, incubated for 30 min and centrifuged in the microfuge for 2 min. The pellets were washed by centrifugation once with incubation buffer containing 0.1% SDS, once with incubation buffer and once with 0.010 M Tris-HCl, *p*H 6.7 and then extracted with 60 μl 8 M urea, 4% SDS, 0.01 M Tris-HCl, *p*H 7.4, 0.1% β-mercaptoethanol for 30 min at 37 °C and 10 min at 50 °C. The extracts were run on an SDS/urea-polyacrylamide gel.

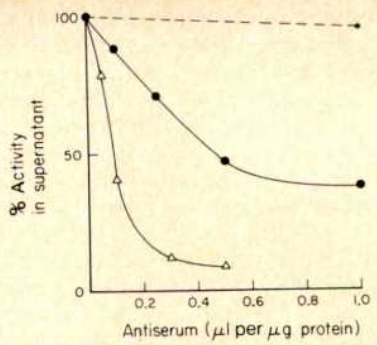


Fig. 3 Immunoprecipitation of complex I from solubilized human kidney submitochondrial particles by antiserum 1 against native beef heart complex I. ●, Antiserum tested on human kidney submitochondrial particles; △, antiserum tested on beef heart submitochondrial particles; ⋆, normal serum tested on human material.

Methods. Submitochondrial particles were prepared from human kidney or beef heart mitochondria and solubilized with 0.5% Na-DOC in the presence of 0.5 M KCl, as described previously[25]. After centrifugation in the 10 × 10 ml rotor of an MSE 65 centrifuge at 25,000 r.p.m. for 40 min, the supernatant was incubated at 4 °C overnight in the presence of 1% Triton X-100. After another centrifugation as above, portions of the supernatant (50 μg protein in 50 μl) were incubated for 1 h at 4 °C with the indicated volumes of antiserum 1 against beef heart complex I or normal serum (with addition of Triton X-100 to a final concentration of 1%). Samples were assayed for NADH-$K_3Fe(CN)_6$ oxidoreductase activity[25] after centrifugation in the conditions described above.

rabbit antisera raised against highly purified native bovine complex I (ref. 24). One such antiserum (antiserum 1) could precipitate ~60% of the NADH-$K_3Fe(CN)_6$ oxidoreductase activity from deoxycholate- and Triton X-100-solubilized human kidney-submitochondrial particles (Fig. 3). The non-precipitated enzyme activity results from an unrelated enzyme with different kinetic properties[25,26] which is more abundant in the kidney (40–50%) than in the heart (10–20%) (C.I.R., unpublished observations). Electrophoretic analysis of the immunoprecipitated human kidney complex I on a 12–16% polyacrylamide gel, in parallel with the beef heart complex I, revealed a profile very similar to that of the beef enzyme, with several recognizable main components in the high-M_r range (>25,000). The human complex I profile diverged from the beef enzyme profile in the low-M_r region, in agreement with previous observations on rat and rabbit complex I (M.W.J.C. and C.I.R., unpublished observations). In immunoblot assays with SDS-denatured human kidney submitochondrial particles, antiserum 1 showed a wide reactivity with human proteins, revealing at least 12 components in the M_r range 10,000–50,000.

The antiserum described above was tested on an SDS mitochondrial lysate from HeLa cells grown for 22 h in the presence of 40 μg ml^{-1} chloramphenicol[27], labelled with ^{35}S-methionine in the presence of 100 μg ml^{-1} cycloheximide and chased in unlabelled medium for 18 h. Only weak bands corresponding to the product of URF4 and to the putative product of URF5 were detected in the autoradiogram of the immunoprecipitate fractionated on a gel, despite the large amount of radioactivity used in this experiment (Fig. 4*a*, lane 3); these bands, however, were specific, because they were absent or much weaker in the control precipitate obtained with normal serum γ-globulins. A more dramatic result was obtained when the same antiserum was tested on a Triton X-100 mitochondrial lysate from the same cells. Six URF products were precipitated in these conditions, that is, the products of URF1, URF2, URF3, URF4, URF4L and URF5, with a minimal degree of contamination by COI, COII and COIII (Fig. 4*b*, lane 6). In the same conditions, antibodies against the COOH-terminal undecapeptide of COII precipitated COI, COII and COIII (Fig. 4*b*, lane 7); these are part of the intact cytochrome *c* oxidase complex

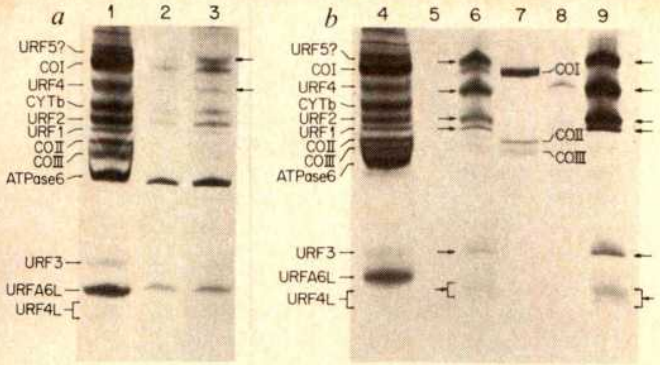


Fig. 4 Antibodies against native bovine complex I immunoprecipitate several URF products. *a*, Immunoprecipitates from an SDS lysate of HeLa cell mitochondria incubated with normal serum γ-globulins (lane 2) or with antiserum 1 against beef heart complex I (lane 3). *b*, Immunoprecipitates from a 0.5% Triton X-100 lysate of HeLa cell mitochondria incubated with normal serum γ-globulins (lane 5), or with antiserum 1 against beef heart complex I (lane 6), or with γ-globulins from an antiserum against the carboxy-terminal undecapeptide of COII (COII-C, ref. 12; lane 7). Lanes 8 and 9 show immunoprecipitates from a 0.2% DOC mitochondrial lysate incubated with normal serum γ-globulins or with antiserum 1 against beef heart complex I, respectively. Lanes 1 and 4, pattern of mitochondrial translation products.

Methods. *a*, Samples of a mitochondrial suspension (10^6 c.p.m., 550 μg protein) from HeLa cells labelled as described in Fig. 2 legend and chased for 2 h were solubilized with SDS and exposed to 50 μl of antiserum 1 against native beef heart complex I or to 500 μg normal serum γ-globulins, as in the experiment described in Fig. 2*a* legend. *b*, Samples of a mitochondrial suspension (2×10^5 c.p.m., 215 μg protein) from cells labelled and chased as described in Fig. 2 legend were solubilized with 0.5% Triton X-100 and immunoprecipitated with 25 μl antiserum 1 (lane 6), or with 250 μg γ-globulins from anti-COII-C antiserum (lane 7), as described in Fig. 2*b* legend. An identical sample (lane 9) was solubilized in 25 μl solution containing 0.2% DOC instead of 0.5% Triton X-100; after 20 min at 4 °C, KCl was added to 0.15 M; after a further 10 min, 65 μl of Zysorbin suspension in incubation buffer (adjusted to 0.15 M KCl) were added and the mixture incubated for 30 min at 4 °C, then spun in the microfuge. The supernatant, after addition of 80 μl of incubation buffer and 25 μl antiserum 1, was incubated for 1 h and then mixed with 125 μl of a 0.5% Triton X-100 mitochondrial lysate (12 mg protein ml^{-1}) from unlabelled cells, then SDS to 0.1% and finally 65 μl Zysorbin. At this stage, the sample contained ~0.015% DOC and ~0.5% Triton X-100. Further treatment of the sample was identical to that of the Triton X-100-solubilized sample. As controls, equivalent portions of the Triton X-100 (lane 5) or DOC-solubilized mitochondrial suspension (lane 8) were incubated with 250 μg γ-globulins from normal rabbit serum. Exposure of the autoradiograms was 10 (*a*) or 12 (*b*) days.

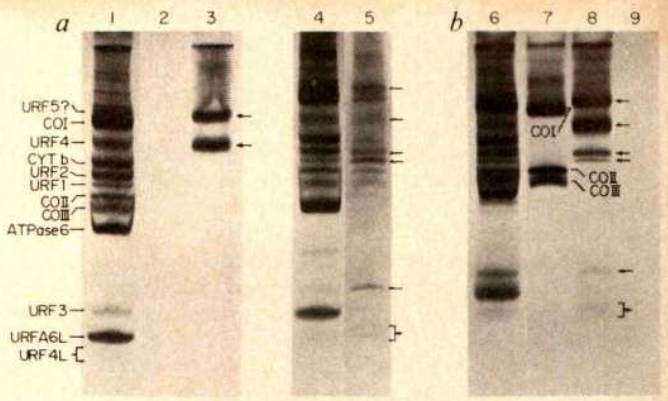


Fig. 5 Effect of detergent treatment (*a*) and labelling conditions (*b*) on the precipitation pattern of URF products by anti-complex I antibodies. *a*, Immunoprecipitates from a 0.5% DOC–1% Triton X-100 mitochondrial lysate incubated with normal serum γ-globulins (lane 2) or with antiserum 1 against beef heart complex I (lane 3). *b*, Immunoprecipitates from a 0.5% Triton X-100 mitochondrial lysate of HeLa cells labelled with ^{35}S-methionine for 2 h, then chased for 2 h (lane 5) or 18 h (lanes 7, 8 and 9), after incubation with antiserum 2 against beef heart complex I (lanes 5 and 8), or with anti-COII-C γ-globulins (lane 7), or with normal serum γ-globulins (lane 9). Lanes 1, 4, 6, pattern of mitochondrial translation products.

Methods. *a*, Two samples of mitochondrial suspension from HeLa cells labelled and chased as described in Fig. 2 legend (3×10^5 c.p.m.; 325 μg protein; lanes 2, 3) were solubilized with 0.5% DOC for 30 min at 4 °C and treated subsequently with 1% Triton X-100 as described previously[25]; after centrifugation at 25,000 r.p.m. in a Spinco 65 rotor for 40 min, the supernatant was incubated with 150 μg normal serum γ-globulin (lane 2) or 15 μl antiserum 1 against beef heart complex I (lane 3). After 2 h incubation at 4 °C and addition of 3 mg unlabelled mitochondrial proteins and SDS to 0.1%, the samples were mixed with 125 μl Zysorbin suspension and further treated as described for the 0.5% Triton X-100-solubilized samples. *b*, Samples of mitochondria from HeLa cells labelled and chased as described in Fig. 2 legend (10^5 c.p.m., 110 μg protein; lane 8), or chased for 2 h after the ^{35}S-methionine pulse instead of 18 h (2×10^5 c.p.m., 190 μg protein; lane 5), were solubilized with 0.5% Triton X-100 and immunoprecipitated with 25 μl (18 h chase) or 50 μl (2 h chase) of antiserum 2 against beef heart complex I, as described in Fig. 2 legend. Equivalent portions of the mitochondrial lysate from 18-h chased cells were incubated with 250 μg γ-globulins from anti-COII-C antiserum (lane 7) or from normal serum (lane 9). Exposure of both autoradiograms in *b* was for 20 days. In a repetition of the immunoprecipitation experiment with 2-h chased material, the normal serum control showed bands of COI, COII and COIII of equal intensity to those of the antiserum-precipitated sample.

(P.M. *et al.*, in preparation). No URF products were observed in the precipitate obtained with normal serum γ-globulins (an independent control experiment showed that complete normal serum gave the same results as normal serum γ-globulins). From the densitometer tracing of the autoradiogram and the methionine content of the various URF products, the labelled products of URF5, URF4, URF2, URF1, URF3 and URF4L in the immunoprecipitate had estimated relative molar concentrations of ~5, 5, 2, 1, 1 and 0.6. In a control experiment, the labelling of all the immunoprecipitated components was sensitive to chloramphenicol.

A more effective precipitation of all six URF products by anti-complex I antibodies was obtained when 0.2% potassium deoxycholate (DOC) in the presence of KCl was used to solubilize the mitochondrial membranes (Fig. 4*b*, lane 9). In these conditions, the relative molar concentrations of the labelled URF products in the immunoprecipitate were similar to those observed in the Triton X-100-solubilized sample (~3, 3, 2, 0.7, 1 and 0.5 for the six polypeptides, respectively). In this last experiment, the solubilized complex I had been exposed to 0.4–0.5% Triton X-100 during the immunoprecipitation and subsequent washing steps (see Fig. 4 legend). Similar results were obtained in another experiment involving exposure of complex I to 0.2% DOC in the absence of Triton X-100 throughout the entire procedure. More drastic conditions of detergent treatment, namely solubilization with 0.5% DOC in the presence of 0.5 M KCl and subsequent treatment with 1% Triton X-100, seem to disrupt the integrity of the complex containing the human URF products. These conditions have been shown previously to leave most of the bovine complex I intact, but to cause a loss of NADH–ubiquinone oxidoreductase activity, although not of NADH–$K_3Fe(CN)_6$ oxidoreductase activity[25]. When the anti-complex I antiserum was tested on mitochondrial membranes treated as outlined above, only the URF4 and URF5 products were detected in the immunoprecipitate (Fig. 5*a*, lane 3); a minimal amount of URF2 and URF1 products was visible in the autoradiogram only after long exposure.

Another factor playing a considerable part in the immunoprecipitation pattern of the URF products are the labelling

conditions. When immunoprecipitation tests were performed on a Triton X-100 mitochondrial lysate from HeLa cells that had been subjected to a short (2 h) chase instead of an 18-h chase, the concentration of URF5 and 4 products precipitated was more than an order of magnitude lower and the amount of URF2 three- to fourfold lower than with material chased for 18 h (compare lanes 5 and 8, Fig. 5). This observation suggests that the incorporation of the newly synthesized URF products into complex I is slow. The experiments of Fig. 5*b* were performed with a different antiserum against bovine complex I (antiserum 2) from that used in the experiments described previously; this antiserum did not precipitate complex I from solubilized human kidney submitochondrial particles, although it reacted with four polypeptides in immunoblot assays. This antiserum, although less effective than antiserum 1, gave a cleaner immunoprecipitate from Triton X-100-solubilized mitochondria than antiserum 1 (compare Fig. 4, lane 6 with Fig. 5, lane 8).

Fraction enriched in six URF products

The results described above strongly suggest that the human respiratory-chain NADH dehydrogenase contains polypeptides encoded in six of the mtDNA URFs. To obtain independent evidence, a mixture of ^{35}S-methionine-labelled HeLa cell mitochondria and human heart mitochondria was subjected to the fractionation scheme which has been used to purify NADH–cytochrome *c* reductase (complex I–III of the respiratory chain) from beef heart mitochondria[28]. The cytochrome *c* oxidase complex from human heart could not be fractionated in the same way as the complex derived from beef heart; as a result, COI, COII and COIII contaminated all fractions to various degrees. Despite this and the constraints imposed on the amount of unlabelled material usable for purification by the requirement for gel analysis and autoradiography, we obtained two fractions (P_5 and P_6, Table 1) enriched in NADH–Q_1 and NADH–$K_3Fe(CN)_6$ oxidoreductase activities. These fractions were essentially free of H^+-ATPase complex, inferred from the almost complete absence of the ATPase 6 and URFA6L polypeptides (Fig. 6). Densitometric measurements showed that ~90% of the ATPase 6 and URFA6L polypeptides remained in the supernatant in the early fractionation step in which complexes I, II and III are precipitated. Fractions P_5 and P_6 were also free of complex II, assayed by succinate dehydrogenase activity. The two fractions were enriched greatly in the six URF products (compare lanes P_5 and P_6 with the marker lane M containing the starting material in Fig. 6). This enrichment could not be correlated with the presence of either cytochrome b–c_1 complex or complex II; the P_6 supernatant, which contained most of the cytochrome *b* polypeptide (estimated ~80% by densitometry) and almost all the succinate dehydrogenase activity of the $P_6 + P_6$ supernatant fractions, showed only a small amount of URF products (estimated to be 10–15%; Fig. 6). Similarly, the enrichment in URF products of P_5 and P_6 could not be attributed to the presence of cytochrome *c* oxidase in these fractions, because both biochemically purified[22] and immunoprecipitated cytochrome *c* oxidase (P.M. *et al.*, in preparation) are totally devoid of URF products. Therefore, it seems that, among the five

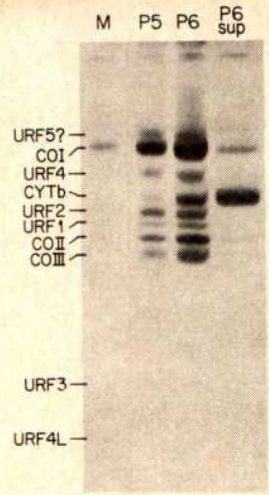


Fig. 6 Distribution of mitochondrial translation products among submitochondrial fractions. P_5, P_6 and P_6 supernatant (P_6 sup) are as defined in Table 1. M, Starting material.
Methods. A mixture of mitochondria from HeLa cells labelled as described in Fig. 2 legend (1.6×10^7 c.p.m., 17 mg protein) and human heart mitochondria (200 mg protein) was subjected to a fractionation scheme for the isolation of NADH–cytochrome *c* reductase (complex I–III)[28]. Equivalent amounts of protein (6.5 μg) of various fractions were run on an SDS/urea polyacrylamide gel.

enzymatic complexes of the oxidative phosphorylation system, the only one whose enrichment can be correlated with the enrichment of P_5 and P_6 in URF products is complex I. From densitometric measurements on the autoradiogram of a gel in which equal amounts of radioactivity of the various samples were analysed, P_5 was estimated to be enriched relative to the starting material ~7-fold in URF4 product and ~5-fold in URF2 and URF1 products; P_6 was enriched ~11-fold in URF4 product and ~7- and 11-fold, respectively, in URF2 and URF1 products. Therefore, the enrichment in URF products in P_5 and P_6 is similar to the enrichment in NADH–Q_1 and NADH–$K_3Fe(CN)_6$ oxidoreductase activities. The deviations from perfect proportionality between NADH dehydrogenase activities and enrichment of URF products in different fractions results from an underestimation of enzyme activities in P_5 and P_6, because of the decrease in solubility of the enzyme that occurs during purification.

Discussion

The conclusion that URF1, URF2, URF3, URF4, URF4L and probably URF5 encode components of the respiratory-chain NADH dehydrogenase is based on four lines of evidence: (1) The products of these URFs are co-precipitated from a Triton X-100 lysate of HeLa cell mitochondria by anti-peptide antibodies specific for individual URF products, especially by anti-URF4L, which suggests that they are components of a complex. (2) Antibodies raised against highly purified bovine complex I react weakly, though specifically, with two URF

Table 1 Enzyme activities of mitochondrial fractions

Fraction	NADH–ferricyanide oxidoreductase (μmol NADH oxidized per min per mg protein)[35]	NADH–Q_1 oxidoreductase (μmol NADH oxidized per min per mg protein)[35]	Succinate dehydrogenase (μmol succinate oxidized per min per mg protein)[36]
Mitochondria	2.7	0.04	0.02
P_5	11.0	0.14	ND
P_6	20.0	0.13	ND
P_6 supernatant	10.0	0.07	0.34

The P_5 fraction is equivalent to complex I–III of Hatefi and Stiggall[28], except that 6.4 ml of 50%-saturated NH_4OAc were added to the previous supernatant per 100 ml of supernatant. The P_6 fraction was obtained from the P_5 supernatant by addition of 6.4 ml of 50%-saturated NH_4OAc per 100 ml of supernatant and pelleting. ND, not detectable at the protein concentration used in the assays.

products in an SDS mitochondrial lysate, that is, with the product of URF4 and the putative product of URF5. (3) The antibodies against bovine complex I precipitate the same six URF products from a DOC or Triton X-100 mitochondrial lysate. (4) A submitochondrial fraction enriched in NADH-Q_1 and NADH-$K_3Fe(CN)_6$ oxidoreductase activities is also enriched in the products of the six URFs.

The URF4-, URF2- and URF3-specific anti-peptide antibodies were less effective than the URF4L-specific antibodies in co-precipitating the other URF products. This may result from a dissociation of the URF product(s) from the complex induced by a destabilizing effect of the antibodies, as has been reported for another mitochondrial complex[29], or to a poor accessibility of the peptide in the complex. The lower capacity of antibodies against native complex I to precipitate URF products from an SDS mitochondrial lysate than from a DOC or Triton X-100 lysate may be because of poor recognition of the SDS-denatured polypeptides by the antibodies, or the effect of SDS on antibody reactivity. Further, it is probable that antibodies against extramitochondrially synthesized components of complex I are also involved in precipitating the complex.

The results described above clearly indicate that the products of URF1, URF2, URF3, URF4, URF4L and the putative product of URF5 are associated closely with complex I. Because this association withstands a variety of detergent treatments, as well as the fractionation procedure for separation of the complexes of the oxidative phosphorylation system, the most plausible interpretation is that the URF products are themselves part of complex I. The enzymatic purity of the complex I preparations used to make the antisera[24] and the evidence indicating that the polypeptides found in complex I preparations are indeed true constituents of the enzyme[25] strongly support this interpretation. It is possible that at least some of the URF products are part of the shell of hydrophobic proteins surrounding the catalytic subunits[20,21]. The estimated M_rs of the URF products discussed here[23], 51,000 (URF5), 39,000-36,000 (URF4), 25,000 (URF2), 24,000 (URF1), 6,000 (URF3) and 3,500 (URF4L), are in reasonable agreement with the reported M_rs for some of the proteins of the insoluble fraction and of the iron-protein fragment of the bovine complex I (ref. 20). We propose that the designations of URF1, URF2, URF3, URF4, URF4L and URF5 be changed to ND (for NADH dehydrogenase) 1, ND2, ND3, ND4, ND4L and ND5, respectively.

In previous work, Hare and Hodges[19] failed to detect any mitochondrially synthesized polypeptide in complex I solubilized from a mixture of unlabelled rat liver mitochondria and labelled rat hepatoma mitochondria and precipitated by antibodies against beef heart complex I. But the labelling conditions used by those authors (2-h exposure of the cells to ^{32}S-methionine without subsequent chase) would allow only very little incorporation of the mitochondrially synthesized, ^{35}S-methionine-labelled subunits into complex I. Further, the authors solubilized complex I from submitochondrial particles with 0.5% DOC and 1% Triton X-100, conditions that we have observed to cause the almost total loss of the four smallest URF products from the HeLa cell immunoprecipitated complex I.

In a more recent investigation[30], the analysis of the mitochondrial translation products and enzyme complexes in a Chinese hamster lung cell mutant, which shows a great decrease in NADH dehydrogenase and cytochrome *c* oxidase activities resulting from an apparently single nuclear mutation, led to the suggestion that complex I contains one or more mtDNA-encoded components[31,32]. The present work provides strong direct evidence for this suggestion.

Functional and evolutionary implications

Yeast mtDNA does not seem to contain any gene homologous to the animal mtDNA URFs that encode components of NADH-ubiquinone oxidoreductase. The NADH dehydrogenase region of the respiratory chain, however, exhibits functional and structural differences in *Saccharomyces* strains compared with the mammalian counterpart; in particular, it lacks site 1 energy coupling[33]. These differences could conceivably result from or be correlated with the absence of some or all of the polypeptides homologous to the animal mtDNA URF products from the yeast complex. On the other hand, it is quite possible that some or all of these polypeptides are encoded in the nucleus and imported from the cytoplasm in yeast. Note that, in contrast to *Saccharomyces* mtDNA, *A. nidulans* mtDNA contains four reading frames that have a convincing amino-acid sequence homology to human URF1, URF3, URF4 and URF5 (ref. 9); a gene homologous to URF1 has been found in *N. crassa* and in *Zea mays*[9]. Genes having a small but significant amino-acid sequence homology to human URF1, URF4 and URF5 and to human URF4 and URF5, respectively, also occur in the kinetoplast maxicircle DNA of *Trypanosoma brucei*[10] and of *Leishmania tarentolae*[11].

It will be of interest to see whether the variation in the complement of mtDNA-encoded subunits in the respiratory-chain NADH dehydrogenase of different organisms is correlated with functional differences. The finding of a variability in genetic control of NADH-ubiquinone oxidoreductase in different organisms extends similar observations concerning the genetic determination of the H^+-ATPase complex[34] and has interesting implications for the evolution of the mitochondrial genome. The evolutionary processes that have led to the segregation of mitochondrial and nuclear genes involved in mitochondriogenesis apparently have followed different paths and progressed to a different extent in different organisms, producing the variety of patterns observed today.

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1. Anderson, S. *et al. Nature* **290**, 457-465 (1981).
2. Bibb, M. J., Van Etten, R. A., Wright, C. T., Walberg, M. W. & Clayton, D. A. *Cell* **26**, 167-180 (1981).
3. Anderson, S. *et al. J. molec. Biol.* **156**, 683-717 (1982).
4. Pepe, G. *et al. Biochem. Int.* **6**, 553-563 (1983).
5. Clary, D. O. & Wolstenholme, D. R. *Nucleic Acids Res.* **11**, 6859-6872 (1983).
6. Clary, D. O., Wahleithner, J. A. & Wolstenholme, D. R. *Nucleic Acids Res.* **12**, 3747-3762 (1984).
7. DeBruijn, M. H. L. *Nature* **304**, 234-241 (1983).
8. Davies, R. W. *et al.* in *Mitochondrial Genes* (eds Slonimski, P., Borst, P. & Attardi, G.) 405-410 (Cold Spring Harbor Laboratory, New York, 1982).
9. Scazzocchio, C., Brown, T. A., Waring, R. B., Ray, J. A. & Davies, R. W. in *Mitochondria 1983. Nucleo-Mitochondrial Interactions* (eds Schweyen, R. J., Wolf, K. & Kaudewitz, F.) 303-312 (de Gruyter, Berlin, 1983).
10. Hensgens, L. A. M. *et al. Nucleic Acids Res.* **12**, 7327-7344 (1984).
11. De La Cruz, V. F., Neckelmann, N. & Simpson, L. *J. biol. Chem.* **259**, 15136-15147 (1984).
12. Mariottini, P. *et al. Cell* **32**, 1269-1277 (1983).
13. Chomyn, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 5535-5539 (1983).
14. Macreadie, I. G. *et al. Nucleic Acids Res.* **11**, 4435-4451 (1983).
15. Attardi, G. *Int. Rev. Cytol.* Suppl. 17 (in the press).
16. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105-132 (1982).
17. Hatefi, Y., Haavik, A. G. & Griffiths, D. E. *J. biol. Chem.* **237**, 1676-1680 (1962).
18. Hatefi, Y. & Stiggall, D. L. *Enzymes* **13**, 175-297 (1976).
19. Hare, J. F. & Hodges, R. *Biochem. biophys. Res. Commun.* **105**, 1250-1256 (1982).
20. Ragan, C. I. in *Subcellular Biochemistry* Vol. 7 (ed. Roodyn, D. B.) 267-307 (Plenum, New York, 1980).
21. Hatefi, Y., Ragan, C. I. & Galante, Y. M. in *The Enzymes of Biological Membranes* Vol. 4 (ed. Martonosi, A.) 1-70 (Plenum, New York, 1984).
22. Hare, J. F., Ching, E. & Attardi, G. *Biochemistry* **19**, 2023-2030 (1980).
23. Ching, E. & Attardi, G. *Biochemistry* **21**, 3188-3195 (1982).
24. Heron, C., Smith, S. & Ragan, C. I. *Biochem. J.* **181**, 435-443 (1979).
25. Smith, S. & Ragan, C. I. *Biochem. J.* **185**, 315-326 (1980).
26. Smith, S., Cotting,ıam, I. R. & Ragan, C. I. *FEBS Lett.* **110**, 279-282 (1980).
27. Storrie, B. & Attardi, G. *J. molec. Biol.* **71**, 177-199 (1972).
28. Hatefi, Y. & Stiggall, D. L. *Meth. Enzym.* **31**, 5-10 (1978).
29. Marzuki, S. *et al.* in *Mitochondria 1983. Nucleo-Mitochondrial Interactions* (eds Schweyen, R. J., Wolf, K. & Kaudewitz, F.) 535-549 (de Gruyter, Berlin, 1983).
30. Malczewski, R. M. & Whitfield, C. D. *J. biol. Chem.* **259**, 11103-11113 (1984).
31. Chu, E. H. Y., Sun, N. C. & Chang, C. C. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3459-3463 (1972).
32. Malczewski, R. M. & Whitfield, C. D. *J. biol. Chem.* **257**, 8137-8142 (1982).
33. Ohnishi, T. *Biochim. biophys. Acta* **301**, 105-128 (1973).
34. Attardi, G., Chomyn, A. & Mariottini, P. in *H^+-ATP Synthase: Structure, Function, Regulation* (eds Papa, S., Altendorg, K., Ernster, L. & Packer, L.) 25-40 (ICSU and Adriatica Editrice Bari).
35. Galante, Y. M. & Hatefi, Y. *Archs Biochem. Biophys.* **192**, 559-568 (1979).
36. Hanstein, W. G., Davis, K. A., Ghalambor, M. A. & Hatefi, Y. *Biochemistry* **10**, 2517-2524 (1971).

Identification and comparison of two sequence elements that confer cell-type specific transcription in yeast

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The MATα2 *protein of budding yeast represses a set of genes; if the* MATa1 *protein is also present, a further set of genes is repressed. DNA sequence comparisons reveal a 20-base pair 'operator' sequence that is present in genes repressed by* a1/α2. *A related, but distinct, sequence is found in genes repressed by* α2 *alone.*

CELL type in the yeast *Saccharomyces cerevisiae* is determined by the allele (*MATa* or *MATα*) that occupies the *MAT* locus[1]. Mackay and Manney[2,3] proposed that *MAT* is a regulatory locus controlling the expression of several unlinked genes required for mating or sporulation. A physical comparison of *MATa* and *MATα*[4,5] has revealed that each allele produces two transcripts, (*a1*, *a2*) or (*α1*, *α2*). Strathern *et al.*[6] proposed that the *MATα1* protein is a positive regulator of some of the genes required for *α* mating, whereas *MATα2* is a negative regulator of some of those required for *a* mating. In *a*/*α* diploids, *MATa1* and *MATα2* act together as a negative regulator of genes not required in diploids, including *MATα1* (Fig. 1; see ref. 7 for a recent review).

In several cases this regulation seems to be exerted at the level of transcription; for example, *α2* function is required for the repression of transcription of the *a*-specific gene *STE6* (ref. 8) and is thought to act similarly on other genes involved in producing the *a* mating phenotype, such as *STE2*, *BAR1* and *MFa1* (V.L.M., unpublished results; A. Brake, personal communication). Once an *α* cell has mated with an *a* cell to produce an *a*/*α* diploid, the function of *α2* becomes modified. As well as its function in *α* cells, it now acts in conjunction with the *MATa1* gene product to repress an additional set of genes. Therefore, these genes are repressed only if both *MATa1* and *MATα2* are expressed. Such 'haploid-specific' genes include *MATα1* (refs 4, 9), *HO* (ref. 10) and *STE5* (J. Thorner, personal communication and V.L.M., unpublished results). Thus, the *α2* gene product represses one set of genes in *α* cells but an additional set of genes in *a*/*α* diploids.

Both *MATa1* and *MATα2* show protein sequence homology with the 'homoeo domain'[11], a highly conserved protein sequence found in gene products involved in selecting developmental fates in *Drosophila melanogaster*[12–14]. It has also been located in genes of unknown function in other phyla, including vertebrates[15,16]. In the case of *MATa1*, part of the homoeo domain is strikingly similar to the DNA binding domain of bacterial repressors[17], suggesting that *MATa1* encodes a repressor protein. There is more direct evidence that the *MATα2* gene encodes a DNA binding protein. An *α2*-*β*-galactosidase fusion protein binds specifically to the 5′-flanking DNA of *STE2* and *STE6* (A. Johnson, personal communication).

Here we have identified the DNA sequences recognized by the *α2* and the *a1*/*α2* repression systems, first by finding candidates on the basis of DNA sequence comparisons, and second by demonstrating that the proposed sequences are sufficient to confer the appropriate control on an unrelated promoter. Our proposed *α2* and *a1*/*α2* control sequences are different but related; we discuss the means by which the two sets of targets (*α2* against *a1*/*α2*) are distinguished.

a1/*α2* Control of the *HO* gene

One of the haploid-specific genes repressed by the action of *a1* and *α2* is the *HO* gene[10], which is responsible for initiating mating type switching (ref. 18 and F. Heffron, personal communication). We have therefore investigated the *a1*/*α2* control of this gene.

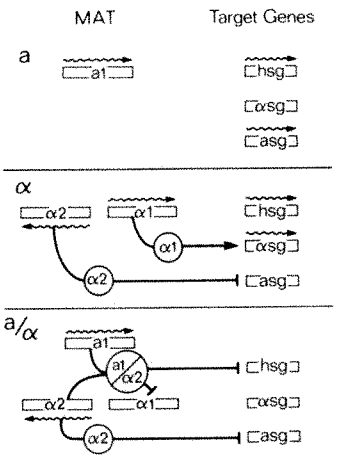


Fig. 1 Mating type control. Left, *MAT* genes: right, unlinked genes which are the targets for their action. Wavy line, gene expression; heavy line, effect of a gene product on the expression of other genes. In an *a* cell, *a*-specific genes (asg) and haploid-specific genes (hsg) are expressed constitutively. In an *α* cell, the asg are repressed by the action of the *α2* gene product, while *α*-specific genes (*α*sg) are activated by the *α1* gene product[6]. When these two cell types mate to produce an *a*/*α* diploid, the *a1* and *α2* gene products together repress the expression of the haploid-specific genes, including *MATα1*.

Two lines of evidence suggest that the sequences that confer *a1*/*α2* repression on *HO* transcription are present in multiple copies distributed widely in the 5′-flanking DNA. The first comes from the deletion analysis by Jensen[19] of plasmids carrying an *HO-lacZ* fusion. A deletion removing all but 296 base pairs (bp) of the DNA flanking the 5′ end of the gene (as measured from the AUG) is fully constitutive, whereas one removing all but 762 bp is fully repressed by the *a*/*α* state. This suggests that *a1*/*α2* control sequences are 5′ to the gene and that they must lie between −296 and −762 from the AUG (Fig. 2). Surprisingly, a deletion (no. 229.102) which removes all the DNA between −172 and −928 is not only transcribed efficiently, but also is fully repressed by the *a*/*α* state when transplaced at the *HO* locus[20]. This suggests that there must be at least two copies of the putative *a1*/*α2* control element, one between −296 and −762, and another either upstream of −928 or in the 3′-flanking sequences.

The distribution of the *a1*/*α2* control elements has also been studied by insertions of the yeast *TRP1* gene at various positions in the *HO* locus. The 850-bp *Eco*RI/*Bgl*II fragment of *TRP1*

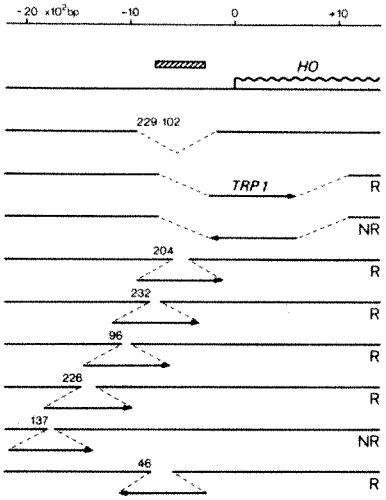


Fig. 2 Redundant a/α control of the *HO* gene. Hatched box, the region defined as important by Jensen[19]; wavy line, ORF of the *HO* gene. The extent of deletion 229.102 is shown. Insertions of the *TRP1* gene into various deletions are also shown; R indicates that in a particular insertion, the *TRP1* gene is repressed in a/α cells; NR indicates that the *TRP1* gene is expressed constitutively. The arrow on the *TRP1* gene indicates the direction of transcription.

Methods. Disruptions of the *HO* gene were constructed and transplaced[20] into the chromosomal *HO* locus of strain K765 (*HMLa MATa HMRa ho::SUP4-o trp1-1 ade2-1 can1-100 his3 leu2-3, 112 ura3*). All insertions fully complemented the *trp1-1* mutation of K765. To test the a/α repression, the transformants were crossed to strain K822 (*HMLa MATα HMRa ho trp1-1 ade2 can1-100 his4 leu2 ura3*) and the TRP$^+$ phenotype of the resulting diploids was scored. In the case of *TRP1* replacing the DNA from −718 to +1,096 (in the same orientation as the *HO* coding sequence), it was shown that the insertion complemented *trp1-1* in a/a and α/α cells, but not in isogeneic a/α cells.

contains all of the coding sequences of the gene encoding (5-P-ribosyl)-anthranilate isomerase but few of its 5′-flanking sequences (only out to −102 from the AUG). Because of its incomplete promoter, the level of expression of this fragment is prone to position effects[21]. Therefore, we have used it as a probe for the regulatory properties of the DNA flanking *HO* by analysing the TRP$^+$ phenotype in *a*, α or a/α *trp*$^-$ cells containing various insertions of *TRP1* in *HO*.

Several *TRP1/HO* insertions (see Fig. 2) have been transferred to the chromosomal *HO* locus using the *SUP4-o* transplacement technique[20]. All the constructions described in Fig. 2 lead to a full TRP$^+$ phenotype in the parent *a* strain. Many of the insertions, however, lose the ability to confer tryptophan prototrophy when an a/α diploid is formed. We have used this specific loss of tryptophan prototrophy as a qualitative measure of the repression of the *TRP1* gene by a/α control. For example, the *TRP1* gene is fully repressed by the a/α state when inserted (in the same orientation as the *HO* coding sequence) as a replacement for the *HO* DNA from −718 to +1,096. In contrast, the gene is constitutive (that is, can confer a TRP$^+$ phenotype to *a*, α and a/α cells) when inserted in the opposite orientation. The simplest interpretation of this result is that *HO* sequences to the left of position −718 can confer a/α repression on the *TRP1* gene and, moreover, they will only do so if joined to its 5′ end. The *TRP1* gene is also regulated when placed (in the same orientation as the *HO* coding sequence) in a number of positions within the interval −300 to −1,450 (see Fig. 2). In contrast, it is not repressed (at least qualitatively) in deletion 137 (−1,737 to −1,777).

These results with *TRP/HO* fusions, taken together with the phenotype of deletion 229.1002 and the results of Jensen[19], suggest that there are several a/α control elements in the interval between −400 and −1,400, and that the 5′ end of the *TRP1* gene must be near one such element to be regulated. Alternatively, there may be fewer elements (although there must be at least two) effective over long distances. Interestingly, an insertion of *TRP1* in the opposite orientation (that is, 5′ end at the 3′ *HO* side) in deletion 46 (−582 to −791) is also under a/α control. This is consistent with the data of Jensen[19] showing that a/α control elements exist in the −296 to −762 interval and suggests that there is one such element to the right of position −582.

In summary, the a/α control of the *HO* gene is redundant, containing two or more control sites within the region −1,777 to −296.

Search for a repeated sequence

In the light of the results described above, the *HO* regulatory region was searched by computer to see whether it contained a repeated sequence motif that satisfied the above criteria. This revealed a sequence motif (T/C)C(A/G)TGTNN(A/T)-NANNTACATCA (Table 1) that is repeated at least 10 times in the 5′-flanking region of the *HO* gene. From the number of occurrences of this motif, its appearance cannot be a chance event; therefore, it must be fulfilling some function. If it is the sequence responsible for $a1/\alpha2$ control, then we would expect to find it it in front of other genes that are repressed by $a1/\alpha2$. Therefore, we have searched the 5′-flanking regions of two genes that are also repressed in the diploid, *MATα1* and *STE5*, for homology with the consensus sequence. At *MATα* there is one occurrence of this sequence (one '$a1/\alpha2$ element'), which lies in the region defined as necessary for $a1/\alpha2$ control[22]. At *STE5* (J. Schultz and J. Thorner, personal communication), there are

Table 1 Examples of the $a1/\alpha2$ element

SEQUENCE	SOURCE	"SCORE"
CAATGTAGAAAAG^{-}ACATCA	MATα1	-13.5
GCTTGTTAATTTACACATCA	STE5 -196	-15.0
TCATGTACTTTTC^{-}GCATCA	STE5 -179	-13.0
CCGCGTTAAAACC^{-}ACATCA	HO -1752	-14.4
TTATGTTAAAAGT^{-}ACATCC	HO -1391	-13.2
GCCTGCGATGAGA^{-}ACATCA	HO -1328	-17.2
TAGAGTGAAAAAGCACATCG	HO -1208*	-18.5
TCATGTATTCATTCACATCA	HO -736*	-10.6
ACATGTCTTCAAC^{-}GCATCA	HO -669*	-14.7
TCGTGTATTTACTTACATCA	HO -576*	-10.0
TCATGTTATTATTTACATCA	HO -411	-8.7
TCATGTCCACATTAACATCA	HO -371	-12.8
GCGTTTAGAACGCTTCATCA	HO -150*	-18.1

CONSENSUS:
TC$^{A}_{G}$TGTNN$^{A}_{T}$NANNTACATCA

The sequences of the $a1/\alpha2$ elements that we have found in the *MATα*, *STE5* and *HO* genes are shown. For the elements marked with an asterisk, the orientation of the element has been reversed (taking the complementary strand) to give a closer fit to the consensus sequence. The 'score' shown is a measure of the fit of each element to the consensus sequence[26]; the larger the negative number, the poorer the fit. Thus, the best fit to the consensus sequence is the *HO* −411 element used in the sufficiency experiment (Fig. 3, insert B). An approximate consensus sequence is shown at the foot of the table. Note that this is an imperfect inverted repeat. Sequence information: *MATα* from ref. 34, *STE5* from J. Schultz and J. Thorner (personal communication), and *HO* from M. Squire and M. Smith (personal communication). The 5′-flanking sequence of the *HO* gene (1,950 bp) was compared with itself and with its complementary strand, using DIAGON[35]; this revealed a number of examples of the putative $a1/\alpha2$ element. The *HO* sequence was compared with the *MATα* intergenic region, revealing the same sequence at *MATα1*: these sequences were aligned to produce a frequency matrix (using GETFRQ) and the *HO* sequence was re-analysed using the ANALYSEQ program suite[26]. This revealed a total of 10 examples of this motif in the *HO* gene and two examples in 705 bp of 5′-flanking sequence at *STE5*. A frequency matrix was then constructed including all known instances of the motif; the aligned sequences are shown in the table. The frequency matrix used to search the EMBL library was derived from these examples, except that the *MATα1* sequence was included three times to give it greater weight. We have re-evaluated all the occurrences we have found, testing their score against a frequency matrix that lacks this arbitrary weighting. Thus, all the 'scores' described here refer to a frequency matrix derived from the set of examples shown in the table.

two such *a1/α2* elements in tandem, ~200 bp upstream from the AUG (Table 1).

The computer search of the *HO* gene also revealed another repeated sequence, CACGAAAA. All occurrences of this sequence, however, are contained in the endpoints of the 229.102 deletion, which still retains *a1/α2* control. This CACGAAA motif seems to be involved in the cell-cycle-dependent transcription of the *HO* gene[23].

Control by the repeated sequence

We have asked whether this sequence ((T/C)C(A/G)-TGTNN(A/T)NANNTACATCA) is able to bring an unrelated promoter under cell-type control. To do this we have used the *CYC1* promoter fused to the *Escherichia coli* β-galactosidase gene[24]. The *CYC1* promoter consists of a TATA box and an upstream element required for efficient transcription, the upstream activating site (UAS). This UAS is sensitive to carbon source, being partially repressed when the yeast are grown on glucose-containing media[25]. We have investigated the effect of inserting an *a1/α2* element into the region between the UAS and the TATA box. We took two different examples of the *a1/α2* element from the *HO* gene and inserted them into a *XhoI* site in the *CYC1* promoter, then asked how these inserts affected the activity of the promoter in different cell types. We find that the parent plasmid and the two insert-containing plasmids all direct the production of similar levels of β-galactosidase activity in isogeneic *a*, *α* and *a*$^-$ cells (when tested on indicator plates; data not shown). If we take an *α* cell carrying the plasmid, we can either mate it to an *a* cell or an *a*$^-$ cell to produce two different diploids. One, the *a/α*, is a normal diploid and will repress its haploid-specific genes. The other, the *a*$^-$*/α* cell, will not repress its haploid-specific genes because *MATa1* is absent. When the β-galactosidase activity of these strains is measured, we find that the parent plasmid is not affected by the difference in mating type (Fig. 3). The plasmids containing an *a1/α2* element, however, show a dramatic reduction in the level of β-galactosidase activity in the *a/α* diploid compared with the *a*$^-$*/α* diploid. This implies that a *CYC1* promoter containing an *a1/α2* element is sensitive to *a/α* control.

This result could arise if the insertion of the *a1/α2* element inactivated the *CYC1* promoter and simultaneously generated a novel *a/α*-sensitive promoter to replace it. To demonstrate that the constructions are still being 'driven' by the *CYC1* UAS, we have tested the carbon source dependence of the plasmids. When grown on raffinose (instead of glucose) the parent plasmid and the two insert-containing plasmids all produce a similar, approximately threefold increase in β-galactosidase (Fig. 3). Therefore, the activity of the *CYC1* UAS is blocked in *a/α* diploids by the presence of an *a1/α2* element between the UAS and the TATA box.

Figure 3 also shows that in *α* cells the insert-containing plasmids produce transcripts identical to those produced by the parent plasmid, but in *a/α* cells no transcript is seen. Therefore, the control is operating at the level of transcription. We conclude that our *a1/α2* element is sufficient to confer cell-type-specific expression on a gene.

Search of other genes

Because the *a1/α2* element seems to be sufficient to confer cell-type-specific repression, we would not expect to find good matches to this sequence in genes that are not under *a/α* control. We have searched the European Molecular Biology Laboratory (EMBL) nucleotide sequence library to see whether there are other genes containing this sequence motif, using the ANALYSEQ program[26]. A matrix was constructed containing the frequencies at which each nucleotide T, C, G or A occurs at each position in the *a1/α2* elements. This matrix was then used to scan DNA sequences to find regions showing a good fit to the consensus sequence.

Some caution must be exercised in interpreting the results of such a search, because the 'goodness-of-fit' observed refers to the consensus sequence that we have constructed and does not

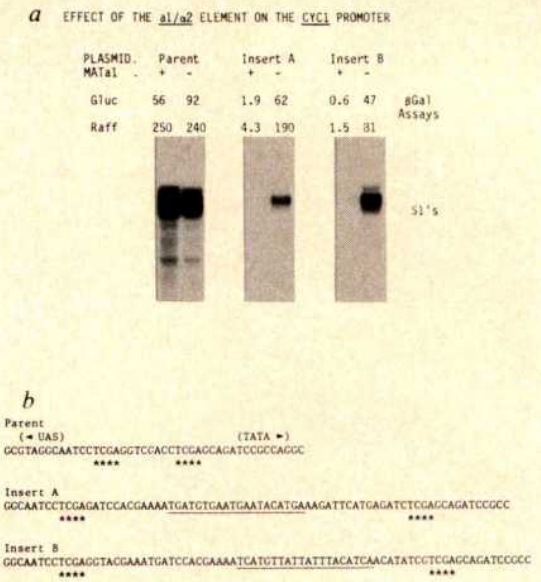


Fig. 3 The *a1/α2* element is sufficient to confer *a/α* control. Two different examples of the *a1/α2* element from the *HO* gene were inserted between the UAS and the TATA box of the *CYC1* promoter driving the β-galactosidase gene. These plasmids were used to transform an *a/α* diploid and the isogeneic *a*$^-$*/α* diploid. *a*, Levels of β-galactosidase (β-Gal) produced in exponentially growing cells are shown, both for raffinose and for glucose as carbon source. Levels of RNA produced by the plasmids were measured by the protection of radiolabelled DNA from S_1 nuclease digestion. *b*, Sequences of the constructions with the *a1/α2* elements underlined. Insert A is *HO* −736; insert B, *HO* −411 (Table 1). Asterisks denote the limits of the inserted fragments.

Methods. DNAs used: Plasmid pHO DNA source was a 4.3-kb *Sau*3A partial fragment and the *Xho* linker mutant 204 derived from it[20]. The *CYC1*-β-galactosidase fusion was pLGΔ312 (a gift of L. Guarente)[36]. The vector for sequencing was M13 mp10 (ref. 37). Cloning of the *a1/α2* elements into the *CYC1* promoter. The *HO* plasmid was digested with *Bam*HI and *Bgl*II, filled in and blunt-end ligated into the filled-in *Xho*I site of pLGΔ-312 (insert A)[36]. *Xho*-linker mutant H204 was digested with *Xho*I and *Nru*I and ligated into the *Xho*I site of pLGΔ312 (insert B). Recombinants were identified by colony hybridization[38] and their identity confirmed by subcloning and sequencing. The *Sma*I/*Bam*HI fragment of pLGΔ-312 carrying the *a1/α2* element insert was cloned into *Hin*cII/*Bam*HI-cut M13mp10. M13 carrying the relevant insert were identified by plaque hybridization and sequenced by the dideoxy-technique. β-Galactosidase assays were performed using the technique of Miller[39]. Cells were collected by centrifugation, resuspended in Z buffer and permeabilized with $CHCl_3$/SDS. After the reaction was stopped with base, cell debris was removed by centrifugation before measuring the A_{420}. Units were measured as $1{,}000 \times A_{420}/A_{660} \times$time (min). RNA samples were taken from cells growing exponentially on glucose and the level of transcription from the plasmid measured as described previously[40]. The copy number of the plasmids did not vary. The DNA probe used is the *Xho*I/*Hin*cII fragment of pLGΔ-312 cloned into *Sal*I/*Sma*I-cut M13mp9 (ref. 37). For the β-galactosidase assays the *MATα* strain used was K822 (Fig. 2); this was mated with either W303-1A (*HMLα MATa HMRa ade2-1 can1-100 his3-11, 15 leu2-3 trp1-1 ura3*) or its derivative RS3 (*HMLα mat*::*LEU2 hmr*::*TRP1 ade2-1 can1-100 his3-11, 15 leu2-3 trp1-1 ura3*). The *mat*::*LEU2* construction is described in ref. 21, and the *hmr*::*TRP1* construction was the kind gift of A. Brand (in preparation). These were transplaced into the yeast chromosome[41]. For the RNA measurements, the strains used were W303-1B (*HMLα MATα HMRa ade2-1 can1-100 his3-11, 15 leu2-3 trp1-1 ura3*) and Y65, which is isogenic, except that it carries a deletion of HMR_E that causes expression of *HMRa1* (A. Brand, manuscript in preparation.)

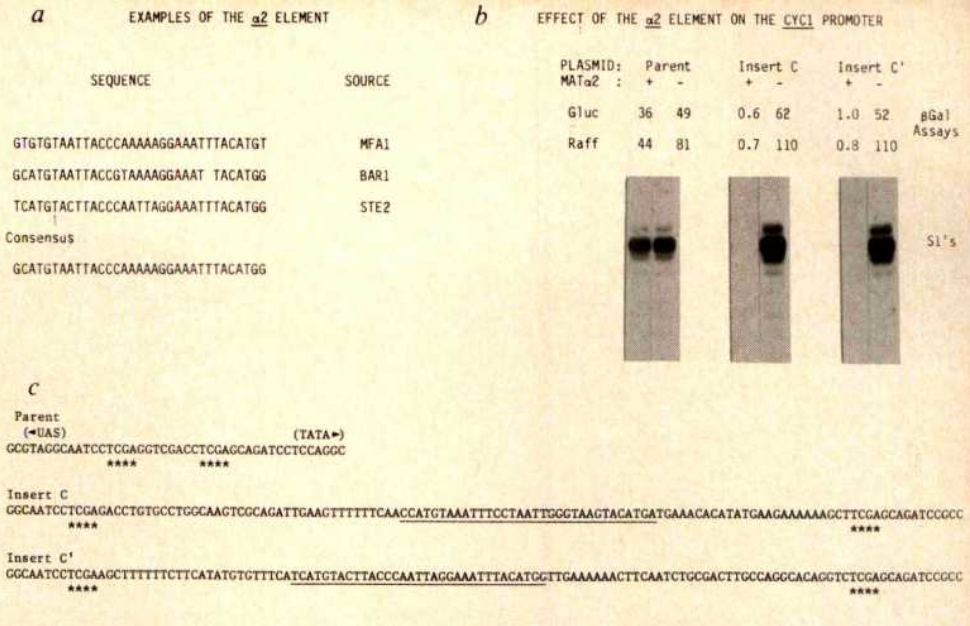


Fig. 4 Sequence of the $\alpha 2$ element and its effect on the *CYC1* promoter. *a*, Sequences of the $\alpha 2$ elements at the *MFa1*, *BAR1* and *STE2* genes. Note that the consensus sequence is an approximate inverted repeat. Sequence information is from A. Brake (personal communication), V.L.M (unpublished results) and this work, respectively. *b*, Effect of the $\alpha 2$ element on the *CYC1* promoter. A 97-bp fragment of the *STE2* gene carrying an $\alpha 2$ element was inserted in both orientations into the *CYC1* promoter. Here we show the levels of β-galactosidase produced by these constructs in isogeneic $a1^-$ and $a1^-\,\alpha 2^-$ cells, both for raffinose and for glucose as carbon source. Levels of RNA produced were measured by the protection of radiolabelled DNA from S_1 nuclease digestion. *c*, Sequences of the constructions with the $\alpha 2$ element underlined. Asterisks denote the limits of the inserted fragments.

Methods. The sequences of the 5′-flanking region of the *BAR1* (690 bp) and *MFa1* (628 bp) genes were compared with each other, and with the 400-bp *Hin*dIII fragment of *STE2* using DIAGON[35]. This revealed the sequences shown here. Sequencing *STE2*:pZV37 (which carries a *STE2*-complementing *Pst*I/*Eco*RI fragment in pUC; V.L.M.) was digested with *Hin*dIII and ligated with *Hin*dIII-cut M13mp10. The sequence was then derived from each end by the dideoxy technique[42]. Inserting a fragment of *STE2* into the *CYC1* promoter. The plasmid carrying the *STE2* gene (pZV37) was digested with *Hin*dIII and *Ava*II and blunt-end ligated into *Xho*I-cut pLGΔ312. Recombinants were identified by colony hybridization and their identity confirmed by subcloning and sequencing. The *Sma*I/*Bam*HI fragment of pLGΔ-312 carrying the $\alpha 2$ element insert was cloned into *Hin*cII/*Bam*HI-cut M13mp10. These recombinants were sequenced. β-Galactosidase assays and RNA measurements were performed as in Fig. 3. The strains used were RS3 (Fig. 3, which is $a1^-\,\alpha 2^-$) and M48 (*HMLα MATα2 matα1::LEU2 hmr::TRP1 ade2-1 can1-100 his3-11, 15 leu2-3 trp1-1 ura3*) (this work). The *MATα2 matα1::LEU2* construction was the gift of L. Breeden (manuscript in preparation).

necessarily reflect exactly what is important for a/α repression *in vivo*.

We have searched 61 kilobases (kb) of yeast DNA sequence in the EMBL nucleotide sequence library and find only one significant match within the 5′-flanking region of any gene. The *MFα1* gene[27] has the sequence CCATGTGAAAAGA-TGCATCT (which would score −15.5 in Table 1) at −230 bp from the AUG. *MFα1* encodes the mating pheromone of the α cell and is an α-specific gene whose transcription is dependent on the *MATα1* gene product[6] (Fig. 1). *MFα1* is not expressed in a/α diploids, partly because *MATα1* is absent. There must, however, be a second a/α-dependent control as *MFα1* is still not expressed in a/α diploids when *MATα1* is expressed constitutively[22]. Our finding that *MFα1* contains a possible $a1/\alpha 2$ control element suggests that this second control is exerted directly by the $a1/\alpha 2$ repressor.

We also find several matches that occur in the open reading frames (ORFs) of genes. We believe that these occurrences of the $a1/\alpha 2$ element do not affect transcription, as transcription is not altered if the $a1/\alpha 2$ element is inserted into the ORF, rather than the promoter, of the *CYC1*-β-galactosidase gene fusion (data not shown). If the matches to the $a1/\alpha 2$ element found in ORFs have no biological effect, then they give a measure of the significance of the matches shown in Table 1. Our search revealed 10 examples of putative $a1/\alpha 2$ elements that would score −16.2 or better in Table 1; therefore, we conclude that such good fits to the consensus sequence should only occur at random once every 6 kb.

$\alpha 2$-Mediated repression

We have shown that the sequence motif (T/C)C(A/G)TGTNN-(A/T)NANNTACATCA is sufficient to confer $a1/\alpha 2$-mediated repression. We know from the biology of yeast mating type that genes carrying this sequence (the haploid-specific genes) are not repressed by $\alpha 2$ alone (Fig. 1); $\alpha 2$-mediated repression must involve a different, perhaps related, DNA sequence. To address this question, we have analysed the DNA sequences of the 5′-flanking regions of three genes that are repressed by $\alpha 2$ alone, looking for sequences specific to these genes which are related to (but different from) the $a1/\alpha 2$ consensus. Using the DIAGON program of R. Staden[35], we have found that *MFa1*, *BAR1* and *STE2* all have a 30-bp sequence in common (Fig. 4*a*). Although in this instance we have only three examples of the sequence, it is clear from the extent of conservation that this sequence has some function. A. Johnson, K. Wilson and I. Herskowitz (personal communication) have also noted this homology.

Two lines of evidence suggest that this 30-bp sequence is involved in $\alpha 2$ control; first, an $\alpha 2$-β-galactosidase fusion protein binds specifically to this DNA sequence (A. Johnson, personal communication). Second, we have shown that a 97-bp fragment containing the putative $\alpha 2$ element from the *STE2* gene can confer $\alpha 2$ control on the *CYC1* promoter. This has been done by inserting the 97-bp fragment between the UAS and the TATA box of the *CYC1* promoter. We then measured the levels of β-galactosidase in isogeneic $a1^-$ and $a1^-\alpha 2^-$ strains; the only genotypic difference between these strains is that the former produces $\alpha 2$ but the latter does not. We find that the plasmid directs β-galactosidase production in the $a1^-\alpha 2^-$ cell, but not in the $a1^-$ cell. Further, the constructions are still under the same carbon source dependence as the parent plasmid, demonstrating that they are being driven by the *CYC1* promoter (Fig. 4*b*). Figure 4*b* also shows that the control is exerted at the level of transcription.

Thus, the 97-bp fragment can bring the *CYC1* promoter under $\alpha 2$ control. Because of the size of this fragment, we cannot be certain whether our consensus sequence alone is reponsible for the regulation, or whether some other sequence on the fragment is involved. We believe that our consensus sequence is responsible because it is the most dramatic homology between the *MFA1*, *BAR1* and *STE2* sequences, and because it has a sequence related to that of the $a1/\alpha 2$ element (see below).

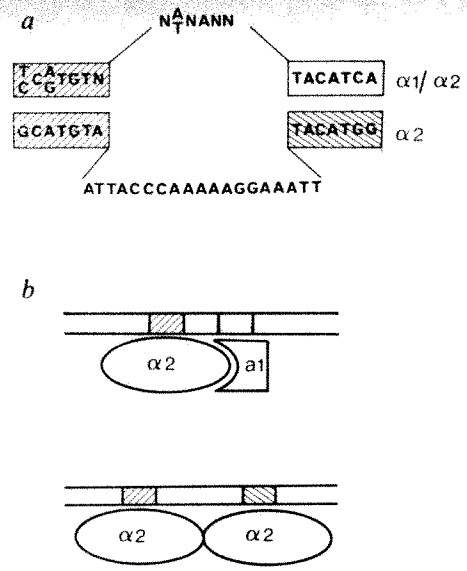


Fig. 5 *a*, Comparison of the *a1/α2* and *α2* elements. The *a1/α2* element (top) is shown aligned with the *α2* element (below) with the common sequence motifs boxed. The TACATG motif is hatched, and the TACATCA motif is stippled. *b*, The combinatorial aspect of mating type control. The *α2* element (bottom) consists of two *α2* half-sites correctly spaced to accommodate the *α2* dimer. The *a1/α2* element (above), in contrast, consists of an *a1* and an *α2* half-site spaced correctly for the 'heterodimer'. It may be that only the spacing of the half-sites is important; alternatively the difference between their sequences may also be important. The critical aspect is that the *α2* half-site in the *a1/α2* element cannot be recognized productively by the *α2* because the other half of the element does not allow cooperative binding by both halves of the *α2* dimer. Hence, the *a1/α2* element is recognized productively only if both *a1* and *α2* are present in the same cell. In such a system, the specificity of an operator depends on three major variables: (1) the presence of short DNA sequences to which particular factors bind specifically; (2) the presence of two or more such sequences in close proximity such that cooperative binding may occur; (3) cooperative binding may be dependent critically on the spacing between the sequences. Only when the correct combination of regulatory proteins is present in a cell is the operator affected.

Discussion

We have identified, by DNA sequence comparisons, two separate sequence motifs that seem to confer two different forms of cell-type-specific repression (*a1/α2* repression in diploids and *α2* repression in *α* cells). To demonstrate that these sequences do confer cell-type-specific repression, rather than some other feature that these genes may have in common, we have excised small fragments containing each of these 'elements' and inserted them into the promoter of an unrelated gene, *CYC1*. In these constructions the *CYC1* promoter comes under the appropriate cell-type control, demonstrating that a promoter can be brought under a particular type of regulation merely by the insertion of the appropriate element into it (Figs 3, 4). Therefore, we believe that the sequence (T/C)C(A/G)TGTNN(A/T)NANN-TACATCA (Table 1) is sufficient to confer *a1/α2*-mediated repression and that the sequence GCATGTAATTACCCAAA-AAGGAAATTTACATGG (or a similar sequence) is sufficient to confer *α2*-mediated repression. This conclusion is consistent with other results (A. Johnson and I. Herskowitz, personal communication) showing that an *α2*-*β*-galactosidase fusion protein binds specifically to the latter sequence *in vitro*.

These two sequences define two 'eukaryotic operator' sequences, in that they are short DNA sequences that repress adjoining genes when present in *cis*. The *a1/α2* element seems to function to full effect only when placed between the UAS (an upstream sequence required for efficient transcription[28]) and the TATA box, but not necessarily next to either promoter element. Hence, it is unlikely to function like a prokaryotic operator, which directly excludes RNA polymerase from binding to the promoter[29]. It could act by blocking the transmission of some signal from the UAS to the TATA box, as suggested by the effect of the *lexA* operator on the *GAL1* promoter[30].

How does the *a1/α2* element differ from the *α2* element such that the former is not recognized by *α2*-mediated repression? The *a1/α2* and *α2* elements have related, but distinct sequences; both are inverted repeats and both carry the same sequence motif (ATGT.... ACAT) in inverted orientation. The first and most marked difference between the two operators is the spacing between the inverted repeats; the common ATGT.... ACAT motif is separated by 11 bp in the *a1/α2* element, but by ~24 bp in the *α2* element. Second, the sequence motifs found in inverted orientation may not be identical (see Fig. 5*a*).

What does the presence of the *a1* gene product in the cell do to extend the action of *α2* to include the *a1/α2* element? Protein sequence data suggest that *a1* is a DNA binding protein; the carboxy-terminal domains of *a1* and *α2* are homologous[11], and we know that the *α2* protein is a sequence-specific DNA binding protein (A. Johnson, personal communication). Furthermore, the homologous region includes the helix-turn-helix motif found in prokaryotic DNA binding proteins[17]. We therefore prefer the model in which *a1/α2* exists on the DNA as a heterodimer, with each protein binding to one half of the *a1/α2* element (to one 'half-site'; Fig. 5*b*).

The model for the specificity of an operator described in Fig. 5*b* involves the combinatorial interaction of regulatory proteins on the DNA; in such a system different proteins that recognize the same DNA sequence (the same half-site) could perform very different functions because of the different protein-protein interactions they can make. Thus, it is significant that the *MATα1* and *MATα2* proteins show homology to the homoeo domain[11], which also seems to conserve the prokaryotic helix-turn-helix motif and is therefore believed to be a DNA binding domain[31]. The genes from *Drosophila* that contain the domain[12-14] have quite distinct functions involved in different aspects of the determination of cell fate. It seems probable that the different functions of these different proteins are encoded by their non-homologous regions. These regions could either specify a second DNA-binding specificity, or, analogously to *a1* and *α2*, they could specify a protein-protein interaction.

We find the idea that homoeo proteins may interact in a combinatorial manner with each other and with other proteins attractive, because (as with *a1* and *α2*) different combinations of these selector genes in different cells would activate different cell-type-specific genes. In this way the combinatorial genetic addresses[32,33] of different cells could be 'read' directly by the promoters of the different cell-type-specific genes.

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1. Mortimer, R. K. & Hawthorne, D. C. *Yeasts* **1**, 385 (1969).
2. Mackay, V. L. & Manney, T. R. *Genetics* **76**, 255-271 (1974).
3. Mackay, V. L. & Manney, T. R. *Genetics* **76**, 273-288 (1974).
4. Nasmyth, K. A., Tatchell, K., Hall, B., Astell, C. R. & Smith, M. *Nature* **289**, 244-250 (1981).
5. Tatchell, K. *et al. Cell* **27**, 25-35 (1981).
6. Strathern, J. N., Hicks, J. B. & Herskowitz, I. *J. molec. Biol.* **147**, 357-372 (1981).
7. Klar, A. J. S., Strathern, J. N. & Hicks, J. B. in *Microbial Development* (eds Losick, R. & Shapiro, L.) 151-195 (Cold Spring Harbor Laboratory, New York, 1984).
8. Wilson, K. & Herskowitz, I. *Molec. cell. Biol.* **4**, 2420-2427 (1984).
9. Klar, A. J. S., Strathern, J. N., Broach, J. B. & Hicks, J. B. *Nature* **289**, 239-244 (1981).
10. Jensen, R., Sprague, G. F. & Herskowitz, I. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3055-3039 (1983).
11. Shepherd, J. C. W., McGinnis, W., Carrasco, A. E., de Robertis, E. M. & Gehring, W. J. *Nature* **310**, 70-71 (1984).
12. McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A. & Gehring, W. J. *Nature* **308**, 428-433 (1984).
13. Scott, M. P. & Weiner, A. J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4115-4119 (1984).
14. Poole, S. J., Kauvar, L. M., Drees, B. & Kornberg, T. *Cell* **40**, 37-43 (1985).
15. Carrasco, A. E., McGinnis, W., Gehring, W. J. & de Robertis, E. M. *Cell* **37**, 409-414 (1984).
16. McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. & Gehring, W. J. *Cell* **37**, 403-408 (1984).
17. Sauer, R. T., Yocum, R. R., Doolittle, R. F., Lewis, M. & Pabo, C. O. *Nature* **298**, 447-451 (1982).

18. Kostriken, R., Strathern, J. N., Klar, A. J. S., Hicks, J. B. & Heffron, F. *Cell* **35**, 167–174 (1983).
19. Jensen, R. thesis, Univ. Oregon (1983).
20. Nasmyth, K. A. *Cell* (submitted).
21. Miller, A. M., Sternglanz, R. & Nasmyth, K. A. *Cold Spring Harb. Symp. quant. Biol.* **49**, 105–113 (1984).
22. Siliciano, P. & Tatchell, K. *Cell* **37**, 969–978 (1984).
23. Nasmyth, K. A. *Cell* (submitted).
24. Guarente, L. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2199–2203 (1981).
25. Guarente, L., Lalonde, B., Gifford, P. & Alani, E. *Cell* **36**, 503–511 (1984).
26. Staden, R. *Nucleic Acids Res.* **12**, 505–519 (1984).
27. Singh, A. *et al. Nucleic Acids Res* **11**, 4049–4063 (1983).
28. Guarente, L. *Cell* 799–800 (1984).
29. Pribnow, D. *Biol. regul. Dev.* **1**, 219–277 (1979).
30. Brent, R. & Ptashne, M. *Nature* **312**, 612–615 (1984).
31. Laughon, A & Scott, M. P. *Nature* **310**, 25–30 (1984).
32. Struhl, G. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7380–7384 (1982).
33. Lawrence, P. A. & Morata, G. *Cell* **35**, 595–601 (1983).
34. Astell, C. R. *et al. Cell* **27**, 15–23 (1981).
35. Staden, R. *Nucleic Acids Res.* **10**, 2951–2961 (1982).
36. Guarente, L. & Mason, T. *Cell* **32**, 1279–1286 (1983).
37. Messing, J. & Vieira, J. *Gene* **19**, 269–276 (1982).
38. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning: A Laboratory Manual*, 315 (Cold Spring Harbor Laboratory, New York, 1982).
39. Miller, J. H. in *Experiments in Molecular Genetics*, 352–355 (Cold Spring Harbor Laboratory, New York, 1972).
40. Nasmyth, K. A. *Nature* **302**, 670–676 (1983).
41. Rothstein, R. *Meth. Enzym.* **101**, 202 (1983).
42. Bankier, A. T. & Barrell, B. G. in *Techniques in Life Sciences* Vol. B508, 1–34 (Elsevier, Amsterdam, 1983).

LETTERS TO NATURE

Combined MERLIN/VLA observations of the superluminal quasar 3C179

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MERLIN[1] (the Multi-Element Radio-Linked Interferometer Network) at Jodrell Bank and the VLA[2] (the Very Large Array) in New Mexico are complementary instruments, representing the latest generation of aperture-synthesis radio telescopes; the VLA comprises 27 antennas, which, in the A-configuration, provide spacings of 0.6–36 km, whereas MERLIN is an array of six antennas with spacings of 6–134 km. Thus, MERLIN provides a factor of four greater resolution than VLA, whereas the shorter spacings of the latter provide essential information on large-scale structure. We present here the first map to be produced by combining data from MERLIN and the VLA. The new map, of the quasar 3C179 (redshift, $z = 0.846$), is a considerable improvement on maps made from the individual arrays and reveals greater detail, particularly in the quasar's jet, which seems to be splitting or disrupting.

3C179 is unusual in that it exhibits superluminal motion in its core[3] yet its extended structure is similar to that of many other classical double or triple sources, whose ejection axes are thought to lie close to the plane of the sky. To study the large-scale structure in detail, 3C179 was observed at wavelength (λ) 18 cm for 24 h using MERLIN, giving a resolution of ~0.25 arc s. The instrument's sensitivity to extended emission on a scale $\geq$3 arc s is limited by the shortest baseline. The VLA was used in the A-configuration to obtain a 20-min extended 'snapshot', giving a reliable representation of structure smaller than ~20 arc s, with a resolution of ~1.2 arc s.

The individual data sets were first calibrated and mapped separately to obtain the maps shown in Fig. 1*a*, *b*, which demonstrate clearly the different capabilities of the two instruments. Because MERLIN maps are produced using closure phases[4] rather than absolute phase, absolute positional information is lost. The MERLIN data were therefore self-calibrated using clean components from the VLA map, effectively shifting the position of the MERLIN map to give the correct phase on MERLIN baselines relative to the VLA. The visibility data from the two instruments were concatenated to give baselines ranging from the minimum VLA spacing of 0.6 to 134 km, the maximum MERLIN spacing. The resultant map is shown in Fig. 1*c*, demonstrating detail not seen in either of the individual maps; this is to be expected because the mapping procedure is a nonlinear process.

The slight discrepancy between the values of peak surface brightness in Fig. 1*b* and *c* is attributable to a small difference between the absolute flux scales of the MERLIN and VLA data. Also, the extended emission in the combined map seems to be

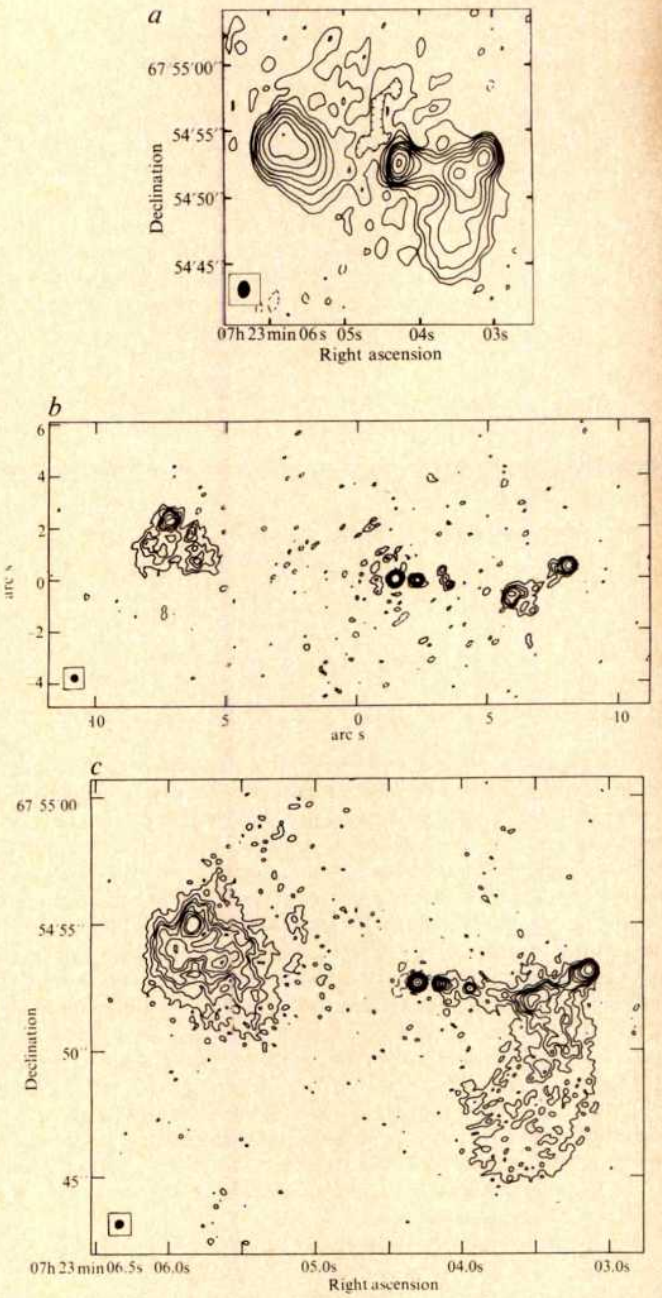


Fig. 1 Maps of 3C179 at λ18 cm. The clean restoring beam in each case is shown in the box in the lower left-hand corner of each map. *a*, VLA map. Contours are plotted at: (−1, 1, 2, 4, 8, 16, 32, 64, 128 and 256) ×0.3% of the peak brightness of 394 mJy per clean beam area. *b*, MERLIN map. Contours are plotted at: (−1, 1, 2, 3, 4, 6, 8, 16, 32, 64) ×0.5% of the peak brightness of 374 mJy per clean beam area. *c*, Combined MERLIN/VLA map. Contours are plotted at: (−1, 1, 2, 3, 4, 6, 8, 12, 16, 32, 64, 128, 256) ×0.3% of the peak brightness of 364 mJy per clean beam area.

weaker than in the VLA map. This is simply a resolution effect; when the combined map is convolved to the resolution of the VLA map, the two give identical results.

Some of the extra detail seen by virtue of the improvement in resolution over that of the λ18-cm VLA map is also apparent in a λ6-cm VLA map of Owen and Puschell[5], which has a resolution of ~0.4 arc s compared with 0.28 arc s for our combined map. The extended structure agrees well with that seen in a λ73-cm MERLIN map[6], which has a resolution of 1 arc s. Thus, we believe that the technique of combining data from the two arrays is reliable.

The new map of 3C179 reveals structural details that are not apparent in the maps from the individual data sets or previous maps. The jet is now clearly seen, and, like many other jets, seems to originate from a bright knot close to the core, which may be a reconfinement shock[7]. The VLBI jet[8] emerges from the nucleus in position angle $(PA) - 88°$; in Fig. 1*c* the arc s jet is seen to bend away from this angle, reaching $PA - 99°$ approximately 3 arc s from the core. A further point of interest is the apparent splitting of the jet into two unequal flows with a possible third towards the north. Ray[9] has suggested that Kelvin–Helmholtz instability modes of the order of $n \geq 3$ will produce a rosette-like cross-section through a jet; if these grow unhindered, they may cause a single jet to split into n jets. Alternatively, the jet may be interacting with local matter at the site of the split, causing deflection and partial disruption. A multi-frequency polarization study is planned to check for depolarization, which may indicate the presence of thermal matter.

The overall structure of the quasar is rather asymmetrical; the relaxed structure of the eastern lobe may indicate that it is either not presently supplied by a jet, or supplied by weak and perhaps poorly collimated ejection from the core. Alternatively, the absence of a counter-jet might be explained by relativistic beaming effects. This would seem unlikely if the ejection axis lies close to the plane of the sky; however, the superluminal motion constrains the ejection axis, at least in the core, to be less than ~29° from the line of sight[6]. In common with other sources exhibiting a one-sided arc s jet, the VLBI jet is on the same side of the nucleus, implying a common cause for the asymmetries of the lobes and jets.

Our map illustrates the value of combining MERLIN and VLA data; the VLA resolution can be improved by a factor of four without the need to use a higher frequency, where steep-spectrum emission will be weaker. By using VLA λ6-cm maps and combined MERLIN/VLA maps at λ18 cm, it may be possible to determine spectral indices and depolarization for source components with greater spatial resolution than hitherto.

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1. Davies, J. G., Anderson, B. & Morison, I. *Nature* **288**, 64–66 (1980).
2. Thompson, A. R., Clarke, B. G., Wade, C. M. & Napier, P. J. *Astrophys. J. Suppl.* **44**, 151–167 (1980).
3. Porcas, R. W. *Nature* **294**, 47–49 (1981).
4. Cornwell, T. J. & Wilkinson, P. N. *Mon. Not. R. astr. Soc.* **196**, 1067 (1981).
5. Owen, F. N. & Puschell, J. J. *Astr. J.* **89**, 932–957 (1984).
6. Browne, I. W. A. *et al. Nature* **299**, 788–793 (1982).
7. Sanders, R. H. *Astrophys. J.* **266**, 73–81 (1983).
8. Porcas, R. W. *Proc. IAU Symp.* **110**, 157–161 (1982).
9. Ray, T. P. *Mon. Not. R. astr. Soc.* **196**, 195–207 (1981).

Periodic minimal surfaces

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A minimal surface is one for which, like a soap film with the same pressure on each side, the mean curvature is zero and, thus, is one where the two principal curvatures are equal and opposite at every point. For every closed circuit in the surface, the area is a minimum. Schwarz[1] and Neovius[2] showed that elements of such surfaces could be put together to give surfaces periodic in three dimensions. These periodic minimal surfaces are geometrical invariants, as are the regular polyhedra, but the former are curved. Minimal surfaces are appropriate for the description of various structures where internal surfaces are prominent and seek to adopt a minimum area or a zero mean curvature subject to their topology; thus they merit more complete numerical characterization. There seem to be at least 18 such surfaces[3], with various symmetries and topologies, related to the crystallographic space groups. Recently, glyceryl mono-oleate (GMO) was shown by Longley and McIntosh[4] to take the shape of the F-surface. The structure postulated is shown here to be in good agreement with an analysis of the fundamental geometry of periodic minimal surfaces.

The F-surface (Schwarz's designation) discussed below would separate the disjunct tetrahedral networks in the cuprite structure (space group Pn3m). The P-surface would separate the two primitive lattices on which the Cs^+ and Cl^- ions in CsCl (space group Pm3m) lie and would be similar to the silicate carcass in rho-zeolite. This P-surface would also closely resemble the surface of zero electrostatic potential in the CsCl structure. The G-surface would separate the two networks postulated by Luzzati and Spegt[5] for strontium myristate (space group Ia3d) and structural correspondence for others of the surfaces can be found.

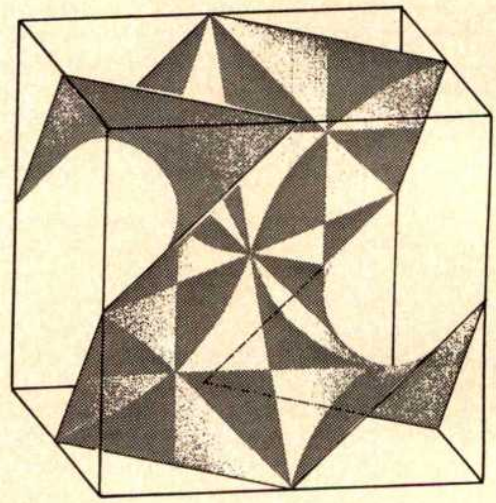


Fig. 1 The cubic unit cell of the F-surface with zero mean curvature[1]. The asymmetric units (48 per cell) are shaded. The space group is Pn3m. In the cuprite Cu_2O structure, or in the double diamond structure of ice VII, this surface would separate the two non-connected networks. In the GMO structure of Longley and McIntosh[4] the surface would be a lipid bilayer with water in the remaining space.

Several of the 18 periodic minimal surfaces described by Schoen[3] (five of which were known to Schwarz) occur as structural elements. Because of their occurrence in lyotropic colloids[4,6], silicates[7–9] and larger-scale biological mineralization[10,11], I have calculated the profiles of several of these surfaces as fundamental structural features.

If the equation for the surface is expressed (in the Monge form) as $z = f(x, y)$, the two-parameter form, then the condition

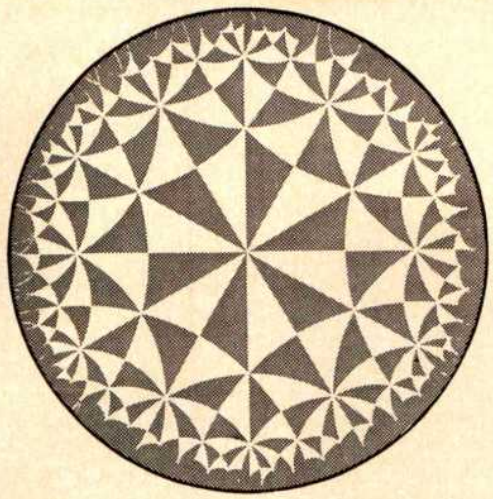


Fig. 2 A stereographic projection of the packing of the same equal triangles as in Fig. 1, but here in the hyperbolic plane, which is a space of constant negative gaussian curvature[13]. The gaussian curvature of the F-surface (Fig. 1) is not constant, but is everywhere negative (except at the umbilical points on the three-fold axes where it is zero). A surface of negative gaussian curvature cannot be mapped onto a plane without tearing or folding, but it can be represented in this way.

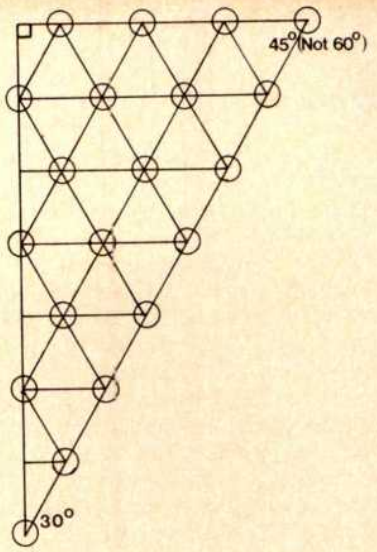


Fig. 3 A possible packing for 12 5/24 molecules of GMO on one side of one of the asymmetric units of the F-surface (Fig. 1). This is an elaboration of the Longley–McIntosh structure and is based on a calculation of the area of the surface. Each molecule would have six neighbours, except that at the 45° vertex (top right), lying on the saddle point where eight triangles meet, which would have eight neighbours. The bilayer (which if planar would be hexagonally close-packed) can be tailored to the F-surface if a disclination occurs where a ring of eight instead of six molecules forms around the saddle point at which the maximum negative gaussian curvature is located.

for zero mean curvature H, is $H = -\text{div}\, n = 0$ (ref. 12), which can be expanded to

$$H = \frac{(1+f_y f_y)f_{xx} - 2f_x f_y f_{xy} + (1+f_x f_x)f_{yy}}{2(1+f_x f_x + f_y f_y)^{3/2}} \qquad (1)$$

where, for example, f_x represents the first partial differential of z with respect to x. This is the Laplace–Young equation.

The gaussian curvature K, the product of the principal curvatures, is given at each point by $K = (f_{xx}f_{yy} - f_{xy}^2)/(1+f_x^2+f_x^2)^2$. The corresponding expression for the area of the element $dx\,dy$ is $dA = (1+f_x f_x + f_y f_y)^{1/2}\,dx\,dy$. An iterative method has been used, calculating the first and second derivatives of eight points nearest on a grid to the given point and adjusting the z-coordinate of the latter to make $H = 0$. K at each point has also been calculated and, as a check, the integrated gaussian curvature, which gives, from $\int K\,dS$, the spherical deficit of the asymmetric unit of area that is bounded by three geodesics. This quantity is also available from the three angles between the geodesics and permits the calculation of the dislocations or disclinations necessary to allow the mapping of a plane net on to the curved surface.

This calculation of z has been performed for the surfaces P, F, CLP, C(P) (Neovius surface) (in Schoen's notation). The calculated areas agree with those found using an analytical method by Schoen (who found 2.3451 for the P-surface and 3.51048 for the C(P) (Neovius surface)), except for the F-surface, where I find 1.9193 whereas Schoen has 2.4177 for the area of surface per unit cube. It is possible that there is here some difference in definition. If the asymmetric unit is taken as a triangle of sides 1/2, $\sqrt{3}/2$, $\sqrt{2}/2$, then the area per unit cube is $3\sqrt{2}/2 = 2.1213$ and the area of these flat panels can clearly be reduced by curving them. If a hyperbolic paraboloid is fitted into a regular tetrahedron, then an area of 1.9212, only slightly greater than our value, is obtained. Schwarz's original diagram of the F-surface (called D by Schoen) is reproduced in modified form in Fig. 1. It can be visualized as the surface separating the two interpenetrating tetrahedral lattices (each of them the diamond network) in the cuprous oxide structure.

From the gaussian curvature we can calculate the mean coordination number (CN) of molecules packed on the curved surface element. The mean CN for a plane surface must be six, but, if the surface is curved synclastically, this can be reduced. For example, if the spherical excess of an equilateral triangle with angles of 72° is 36°, then five of these triangles may meet at a point and we obtain a closed surface of icosahedral symmetry. Similarly, if the surface had a negative curvature so that the spherical deficit of a triangle were 25.71°, then seven triangles, with angles 360/7 = 51.43°, could meet at a point thus tiling an imaginary sphere (a sphere of radius i) of anticlastic curvature.

The spherical deficit for the triangle (with angles 90°, 45° and 30°), which is the unit of pattern for the F-surface, is 15°. The maximum gaussian curvature occurs at the saddle point and gives radii of curvature there of +0.29 and −0.29 (for a unit cube). Figure 2[13] shows a stereographical projection of a packing of these triangles with the same local connectivity as in the F-surface, but in the hyperbolic plane with a constant gaussian curvature. Bonnet's theorem, "that with every surface of constant mean curvature $(2A)^{-1}$ there is a parallel surface of constant gaussian curvature at a distance A from it", is relevant here, but the two surfaces are infinitely far apart (because the minimal surface has a zero mean curvature). The F-surface maps onto the hyperbolic plane in multiple layers joined at the umbilical points.

The best example of a molecular structure taking the form of the F-surface is that of GMO with water, studied by Longley and McIntosh[4]. Their structure is cubic, of space group Pn3m, with $a = 105$ Å. An F-surface of this dimension would have an area of 21,160 $Å^2$ per unit cell. Assuming, as these authors suggest, a 60% GMO content by weight, there should be (with a relative molecular mass 372.55 and a density for neat GMO of 1.028) some 1,140 molecules of GMO per cell. If they form a bilayer, as seems probable, then the area per molecule should be 37 $Å^2$, which agrees with the value of 37.9 ± 0.2 $Å^2$ found by White[14] for planar bilayers of GMO in the liquid-crystalline state.

There are 48 unit triangles (repeated by reflection to give eight to each saddle contained in a $1/2 \times 1/2 \times 1/2$ tetrahedron) of surface per unit cell (each with a spherical deficit of 15°) (Fig. 1). Each should carry ~11.8 molecules on each face to give a bilayer structure.

A disclination of +60° in a hexagonal network corresponds to one 5-fold instead of a 6-fold node. Thus, 12 such units are necessary to make up the 720° total if a sphere is to be covered. A disclination of −60° corresponds to the introduction of an extra segment giving a 7-fold node and a total disclination of 120° would correspond to one 8-fold node or two 7-fold nodes. In the F-surface structure, with a deficit of 15° per asymmetric unit, there is a total disclination strength of 120° for the eight units associated with each saddle point, which is where the maximum curvature is located. Thus, in the Longley-McIntosh GMO structure there should be about one dislocation, in a quasi-planar arrangement otherwise hexagonally close-packed, with an 8-fold instead of a 6-fold node associated with each of the six saddle points per unit cell. Because there are about 11.8 molecules in the asymmetric unit, this might correspond to the idealized packing shown in Fig. 3, where there would be 12 5/24 molecules per asymmetric unit.

The crystallographic structure factors $F(h, k, l)$ for several of the surfaces have also been calculated, because they should also be useful geometrical invariants. For the F-surface the first 10 values are (normalized to $F(0, 0, 0) = 1.9193$ (the total area per cell) and including no atomic scattering curves: $F(0, 0, 0)$, 1.9193; $F(1, 1, 0)$, 0.4775; $F(1, 1, 1)$, 0.4866; $F(2, 0, 0)$, −0.2564; $F(2, 1, 1)$, 0.2129; $F(2, 2, 0)$, 0.2578; $F(2, 2, 1)$, 0.2789; $F(3, 1, 0)$, −0.1877; $F(3, 1, 1)$, −0.0946; $F(2, 2, 2)$, 0.2960; $F(3, 2, 1)$, 0.0952; $F(4, 0, 0)$, 0.1458.

We would expect to encounter the physical occurrence of other of the periodic minimal surfaces, particularly in liquid-crystal systems, although the F-surface discussed here has probably the smallest maximum curvature. We consider it a useful objective to calculate the profiles, curvatures and structure factors of all these periodic minimal surfaces, because they are geometrical invariants to which a number of physical structures can often be usefully referred.

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1. Schwarz, H. A. *Gesammelte Mathematische Abhandlungen* Vols 1, 2 (Springer, Berlin, 1890).
2. Hilbert, D. & Cohn-Vossen, S. *Geometry and the Imagination*, 271 (Chelsea, New York, 1952).
3. Schoen, A. H. *NASA Tech. Note* D-5541 (1970); *Not. Am. Math. Soc.* **16**, 519 (1969); **15**, 929 (1968); **15**, 727 (1968); **14**, 661 (1967).
4. Longley, W. & McIntosh, T. J. *Nature* **303**, 612-614 (1983).
5. Luzzati, V. & Spegt, P. A. *Nature* **215**, 701-704 (1967).
6. Fontell, K. *Molec. Crystal Liq. Crystal* **63**, 59-82 (1981).
7. Mackay, A. L. *Silicate Membranes as Minimal Surfaces* (Poster/Abstract 73-P1-5a *Abstr. IUC Reg. Conf.* Copenhagen, 1979).
8. Andersson, S. *Angew. Chemie* **22**, 69-81 (1983).
9. Andersson, S., Hyde, S. T. & Schnering, H. G. von, *Zeit. f. Krist.* **168**, 1-17 (1984).
10. Donnay, G. & Pawson, D. L. *Science* **166**, 1147-1150 (1969).
11. Nissen, H-L. *Science* **166**, 1150-1152 (1969).
12. Weatherburn, C. E. *Differential Geometry* Vols 1, 2 (Cambridge University Press, 1939).
13. Coxeter, H. S. M. *Introduction to Geometry*, 285 (Fig. 15.8c) (Wiley, New York, 1961).
14. White, S. H. *Biophys. J.* **23**, 337-347 (1978).

Residence-time distributions in regions of steady-flow systems

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Questions often arise in the applied sciences concerning the transport of material through a system[1-4] (for example, a chemical plant, the cardiovascular system or a river basin). Conserved particles such as molecules, blood corpuscles or colloidal particles passing through the system spend different times in the system even when the flow is steady. The theory of residence times[1,2] may be used to relate tracer studies to theoretical or empirical models. Existing theory mainly concerns complete systems, but a few results have been established[2,5-7] concerning the times that particles spend in a specific region (for example, a particular phase in a chemical reactor, an organ or a backwater) while passing through the system. I show here that the distribution of these 'region residence times' may be calculated from a system model by using a formalism in which the volumes of all the other regions are reduced to zero, allowing the region distribution to be calculated by well-established methods[1,2] that apply to systems.

Most existing theory is confined to systems with closed inlets and outlets. A closed inlet or outlet is one where the particles move solely by bulk flow: once particles enter the system they cannot re-emerge, even temporarily, at the inlet, nor, once they have left the outlet, can they re-enter the system. This restriction is needed to avoid ambiguity when a tally is taken of the particles. The residence-time density function $f(t)$ is defined by saying that $f(t)\mathrm{d}t$ is the proportion of particles entering (or leaving) the system that will spend (or has spent) a length of time between t and $t+\mathrm{d}t$ within the system when it is in the steady state.

To see the importance of the distribution of times that particles spend in a region of a system, consider, for example, the times

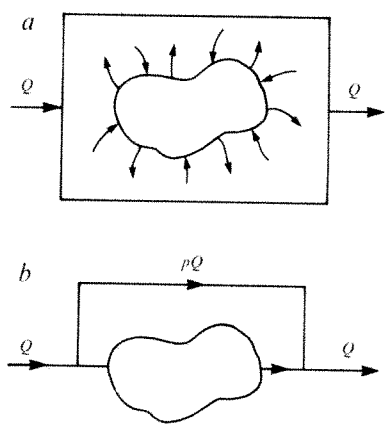


Fig. 1 *a*, A system with a well-mixed region; *b*, the system obtained by using the formalism gives the region residence-time distribution.

that molecules of a drug administered orally spend in a particular organ before being eliminated from the body, or the times that pollutant molecules discharged into a lake spend in a certain zone of the lake before eventually being carried downstream. In these examples there is no need for the region to be closed. A particle may enter a region several times during its journey through the system, or it may bypass the region altogether. Even if the region does have closed boundaries it may be possible for particles to recycle from the region outlet to the region inlet. The residence time in a region is the total time a particle spends in the region between first entering the system and finally leaving it. The residence-time density function (loosely 'distribution') $f_R(t)$ for a region is defined in the same way as the system distribution $f(t)$. Gibilaro[5] has shown that the residence-time distribution for a well-mixed region of a system (Fig. 1*a*) is the sum of an exponential distribution and a spike with zero residence time that represents particles which do not enter the region during their passage through the system. Nauman[6] has shown that the residence-time distribution for an open segment of a

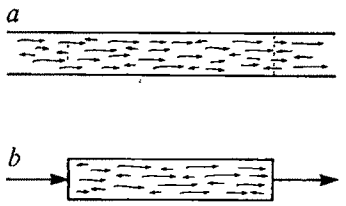


Fig. 2 Axially dispersed piston flow: *a*, showing a segment with open boundaries; *b*, showing the upstream and downstream volumes reduced to zero to find the system that gives the region residence-time distribution.

duct carrying an axially dispersed fluid (Fig. 2*a*) is exactly the same as it would be if the segment were closed. The mean residence time in a region is the ratio of the hold-up in the region to the rate of transmission through the system[7].

Nauman's result was the solution to what had been a perplexing problem. His method was to imagine a particle undergoing a random walk and a clock that stops whenever the particle leaves the segment on which attention is focused and restarts when it re-enters. Residence time is the time the clock records between initial entry and final departure. This provides the clue to deducing the residence-time distribution for an open region. Instead of timing particles with clocks, imagine that each particle is provided with a watch which runs only when the particle is within the region. When a particle leaves the region for the last time, its watch shows the residence time. If, when outside the region, the particles could be induced to travel at infinite speed but along their proper paths, their watches could run continuously. Thus we arrive at the following formalism for deducing the region residence-time distribution from a system model. Reduce the volumes of all regions of the model other than the one of interest to zero and find the residence-time distribution of the resulting model. We can thus establish Gibilaro's and Nauman's results in an extremely simple and straightforward way.

As the portion of the system in Fig. 1*a* outside the well-mixed region is shrunk, particles entering the system reach the well-mixed zone more quickly, return to it more quickly, and so on. At the limit, returning particles return instantaneously. Removing particles from a well-mixed region and instantaneously returning them, to whatever location, has no observable physical effect. Consequently the region residence-time distribution is the same as the system distribution for the system in Fig. 1*b* and is

$$f_R(t) = p\delta(0) + [(1-p)^2 Q/h_R] \exp[-(1-p)Qt/h_R] \quad (1)$$

as Gibilaro[5] showed. The impulse, $p\delta(0)$, represents particles that bypass the region and h_R is the steady-state hold-up of particles there. For models of modest complexity it is usually possible to deduce the bypass fraction p by *ad hoc* methods, but a general procedure is available[8] for network models. For the case of axially dispersed piston flow (Fig. 2*a*), replacing the parts of the system upstream and downstream of the region of interest by regions with no volume gives the closed system of Fig. 2*b* and confirms Nauman's finding[6] that the residence-time distribution for the open region in Fig. 2*a* is the same as it would be if the region were closed.

The formalism can also be used to derive new results. For example, consider a region with a single closed inlet and a single closed outlet embedded within a system (Fig. 3*a*). When the system is shrunk it becomes equivalent to that shown in Fig. 3*b*: it may be possible for particles to bypass the region; other particles either leave after one passage through the region or are recycled. The region residence-time density is

$$f_R(t) = p\delta(0) + (1-p)f_{RS}(t) \quad (2)$$

where $f_{RS}(t)$ is the residence-time density for the recycle system within the broken line in Fig. 3*b*. This density is related to the density of (once-through) sojourn times in the region[2,9].

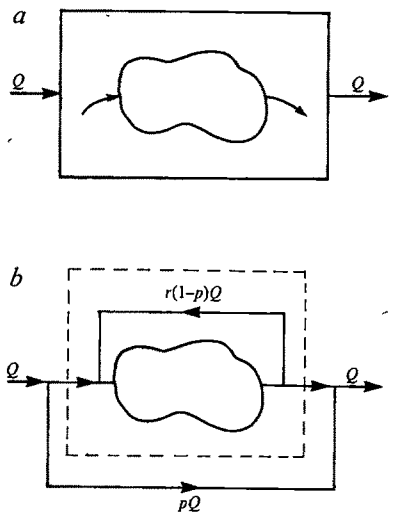


Fig. 3 *a*, A region with a closed inlet and a closed outlet embedded within a system; *b*, the system that gives the region residence-time distribution.

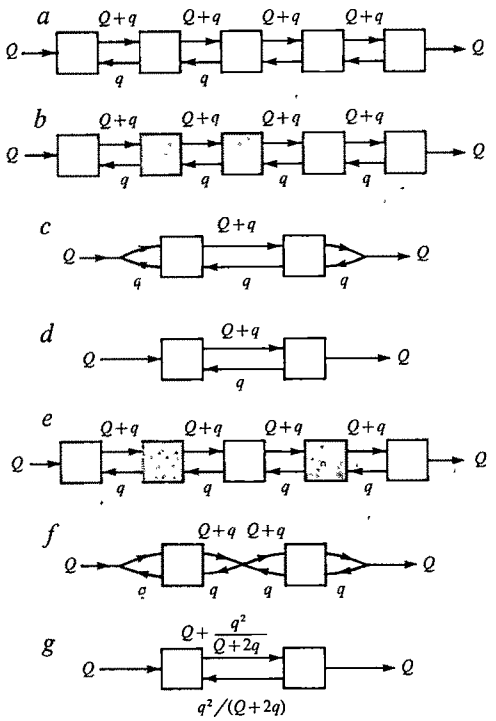


Fig. 4 Sequences of well-mixed zones with backflow: *a*, the basic model; *b*, a region consisting of two adjacent zones; *c*, the system that gives the region residence-time distribution for a region consisting of two adjacent zones according to the formalism; *d*, the system of *c*, further simplified; *e*, a region consisting of two non-adjacent zones; *f*, the system that gives the residence-time distribution for a region consisting of two non-adjacent zones; *g*, the system of *f* further simplified.

Gibilaro[9] has provided a survey of recycle models. The bypass ratio p and recycle ratio r will be reasonably easy to establish for relatively simple models and again the general method[8] can be used for networks.

As a further example, consider the sequence of perfectly-mixed equal-volume zones with backflow shown in Fig. 4*a*. Suppose we wish to know the distribution of residence times in a region that consists of two adjacent zones (Fig. 4*b*). By applying the formalism, the system in Fig. 4*c* is found to have the desired residence-time distribution. This can be simplified

further to give the system in Fig. 4*d*. Application of standard methods[2] shows that the residence-time density is

$$f(t) = (Q/V_R)\sqrt{1+Q/q}\,\{e^{-\lambda_1 Qt/V_R} - e^{-\lambda_2 Qt/V_R}\} \qquad (3)$$

where $\lambda_{1,2} = 2\sqrt{1+Q/q}/(\sqrt{1+Q/q} \pm 1)$ and V_R is the region volume. Backflow cell models have been discussed by others[10-12]: residence-time distributions for regions consisting of several adjacent zones can be deduced from published results[10,11] by using the formalism. We can also find the residence-time distribution for a region consisting of two non-adjacent zones (Fig. 4*e*). When the formalism is invoked, the zone(s) between the two zones comprising the region shrinks into a node (Fig. 4*f*). Of the flow from the left-hand zone to the node, a fraction $(Q+q)/(Q+2q)$ passes on to the right-hand zone and a fraction $q/(Q+2q)$ returns to the left-hand zone. As the latter is returning at once to a well-mixed zone it has just left, it can be disregarded. Thus, the significant left-to-right flow is $(Q+q)^2/(Q+2q)$, which can also be written as $Q+q^2/(Q+2q)$. As the net flow is Q, the significant right-to-left flow is $q^2/(Q+2q)$ (see Fig. 4*g*). It follows by comparing Fig. 4*d* and *g* that the residence-time distribution is given by equation (3), with q replaced by $q^2/(Q+2q)$.

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1. Wen, C Y & Fan, L T *Models for Flow Systems and Chemical Reactors* (Dekker, New York, 1975)
2 Nauman, E B & Buffham, B A *Mixing in Continuous Flow Systems* (Wiley, New York, 1983)
3 Shepherd, C W *Basic Principles of the Tracer Method* (Wiley, New York, 1962)
4 Fischer, H B, List, E J, Koh, R C Y, Imberger, J & Brooks, N H *Mixing in Inland and Coastal Waters* (Academic, New York, 1979).
5 Gibilaro, L G *Chem Engng Sci* 34, 697-702 (1979)
6 Nauman, E B *Chem. Engng Sci* 36, 957-966 (1981)
7 Buffham, B A *Nature* 274, 879-880 (1978)
8 Rubinovitch, M. & Mann, U *A I Ch E Jl* 29, 658-662 (1983)
9 Gibilaro, L G *Chem. Engng Sci.* 26, 299-304 (1971)
10. Roemer, M H & Durbin, L D *Ind Engng Chem Fundamentals* 6, 120-129 (1967)
11 Haddad, A H & Wolf, D. *Can J chem Engng* 45, 100-104 (1967)
12 Mecklenburgh, J C. & Hartland, S *The Theory of Backmixing* (Wiley, Chichester, 1975)

Atmospheric chlorofluoromethanes in the deep equatorial Atlantic

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Waters that leave the surface of the ocean and enter the subsurface circulation contain concentrations of CCl_3F (fluorocarbon-11) and CCl_2F_2 (fluorocarbon-12), which reflects the temporal increases of these industrially produced compounds in the atmosphere. These chlorofluoromethanes (CFMs) are extremely stable in the troposphere and in natural waters, they have no known natural sources, and their histories of release to the atmosphere are fairly well known[1]. The atmospheric distributions of CCl_3F and CCl_2F_2 are not strongly dependent on latitude[2,3], and their surface water concentrations can be expected to come into relatively rapid solubility equilibrium with the atmosphere[4]. Recent advances in analytical techniques[5,6] have made possible the routine use of these CFMs as oceanic tracers on a decadal timescale. The results we report here are from the first detailed surveys of CCl_3F and CCl_2F_2 distributions in the northern and tropical Atlantic Ocean. They show that CFM-bearing waters originating in the region of the Labrador Sea have reached the Equator in a well-defined western-boundary undercurrent located at a depth of about 1.6 km, in the Upper North Atlantic Deep Water. Using a simple dilution model, we calculate that this water has taken about 23 yr to reach the equatorial region, and has been diluted about five-fold by CFM-free waters.

Our measurements were carried out aboard R/V *Knorr* during Leg 7 of the Transient Tracers in the Ocean (TTO) North Atlantic Study (NAS) in September–October 1981, and during the three legs of the TTO Tropical Atlantic Study (TAS) from December 1982 to February 1983. The station numbers and locations of these expedition legs are shown in Fig. 1. Concentrations of CCl_3F and CCl_2F_2 were measured at about 15–30 depths at each station, and in samples of marine air, using shipboard electron-capture gas chromatography. The techniques for measuring CFMs have evolved considerably during the course of this work: the NAS measurements were made using a modified version of the technique used earlier in the North Pacific[5], and the TAS measurements were made by a semi-automated low-blank version of the technique used in the Greenland and Norwegian seas[6]. Seawater samples were collected in Niskin bottles and carefully transferred to 100-cm^3 glass syringes to avoid contact with shipboard air, which was generally contaminated in CFMs. Measured seawater volumes of ~30 cm^3 were injected into a stripping chamber, where the dissolved CFMs were purged by a stream of purified carrier gas and were collected on a low-temperature trap for injection into the gas chromatograph using a Porasil C separating column. Impurities with long elution times were removed using standard pre-column techniques. The peaks were detected by a ^{63}Ni electron-capture detector operating in constant current mode, and amounts were determined by peak height measurement on NAS and by digital integration on TAS. Seawater samples were analysed within a few hours of collection. Measured volumes of clean ambient air (~3 cm^3) drawn from a continuously pumped outdoor sampling line and dried with potassium carbonate on NAS and magnesium perchlorate on TAS were analysed by injection into the same low-temperature sampling system.

The system was calibrated by frequent analyses of compressed air secondary standards in various aliquot sizes. The values we report are given on the Scripps Institution of Oceanography (SIO) 1984 calibration scale, which is based on primary standards with near-atmospheric CCl_3F and CCl_2F_2 concentrations prepared in the laboratory by volumetric dilution. The estimated accuracy of this scale is ~1.5% for CCl_3F and ~0.3% for CCl_2F_2. We have also carried out direct intercomparisons between our standards and those used in the Atmospheric Lifetime Experiment (ALE)[7] which demonstrate agreement within expected combined errors: values reported on the ALE scale are 2.5% higher than the SIO 1984 scale for CCl_3F and 1.9% lower than the SIO 1984 scale for CCl_2F_2.

The precision of the seawater measurements, as estimated from the standard deviation of replicate analyses, is about 4% or 0.02 pmol kg^{-1}, whichever is greater, for both CFMs on NAS, and about 1% or 0.005 pmol kg^{-1}, whichever is greater, for both CFMs on TAS. Systematic sampling and handling errors are estimated to be on the same order.

To assess past surface-water CFM concentrations, we have reconstructed the temporal variations in high-latitude Northern Hemisphere tropospheric CCl_3F and CCl_2F_2 mixing ratios in the same way as we have done for the Greenland and Norwegian seas[6]. For the period since 1976, we have used direct measurements[2,3,8], corrected to the SIO 1984 calibration scale. For 1933–76, we have integrated annual CFM release figures[9], assuming tropospheric residence times of 50 yr for CCl_3F and 90 yr for CCl_2F_2, and have normalized these results to match the direct measurements in 1976. These data show monotonic increases in the tropospheric concentrations of both compounds, with build-up of CCl_3F beginning somewhat later but rising more rapidly, so that there has been a steady increase in the CCl_3F/CCl_2F_2 ratio from zero in 1937 to about 0.57 in 1975. Following the discovery that the CFMs represent a threat to the Earth's ozone layer[10], the percentage rates of increase of both of these compounds have gradually decreased to about 6% per year, and there has been no significant change in the tropospheric CCl_3F/CCl_2F_2 ratio since about 1976.

Each of the four TAS hydrographic sections which cross the western boundary of the tropical Atlantic (Fig. 1) show pro-

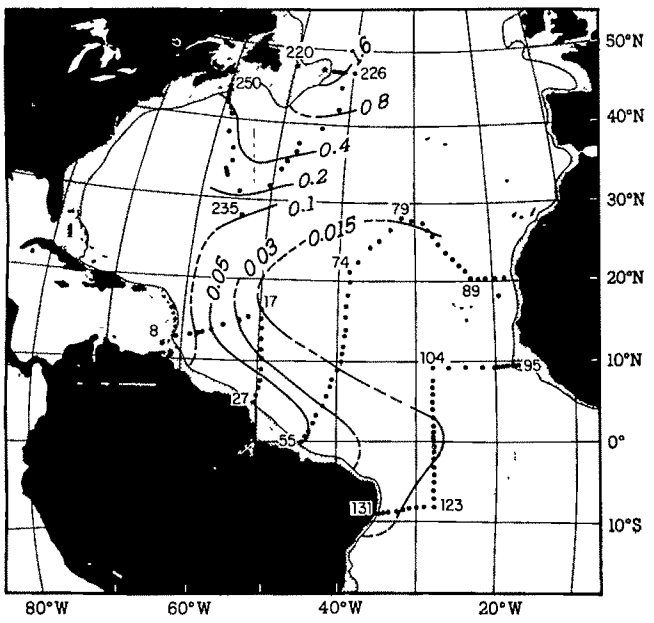


Fig. 1 Station locations and contours of CCl_3F concentrations on the $\sigma_{1.5} = 34.63$ kg m^{-3} isopycnal surface. Stations 220–250 in the northwestern Atlantic are from the NAS expedition and Stations 8–27 and 55–131 in the tropical Atlantic are from the TAS expedition. CCl_3F contours are in units of pmol kg^{-1}, and are spaced at approximately logarithmic intervals in which each successive contour above 0.05 pmol kg^{-1} represents a doubling in concentration.

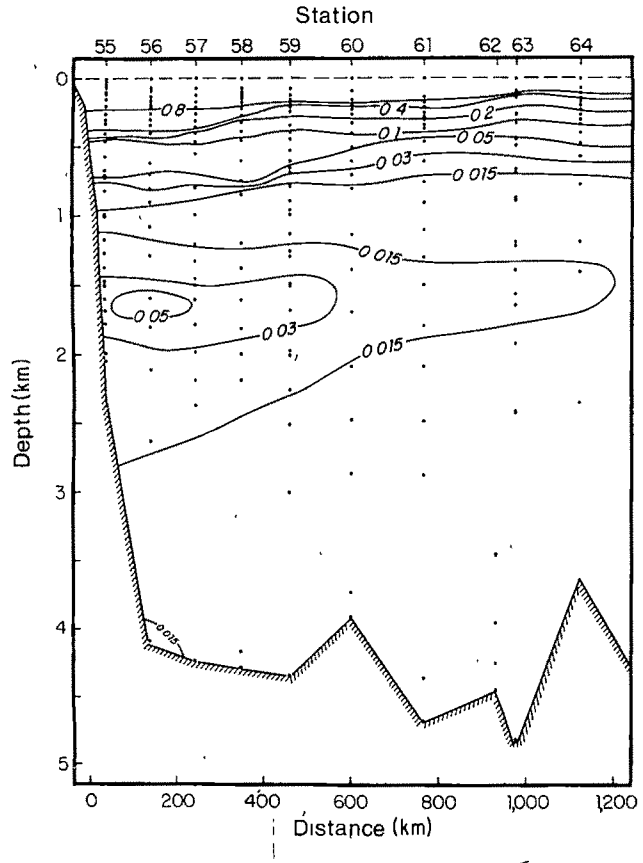


Fig. 2 Concentration of CCl_3F in pmol kg^{-1} on the TAS vertical section extending from Station 55 near the South American continent at the Equator to Station 64 near the Mid-Atlantic Ridge. The contour intervals are as in Fig. 1.

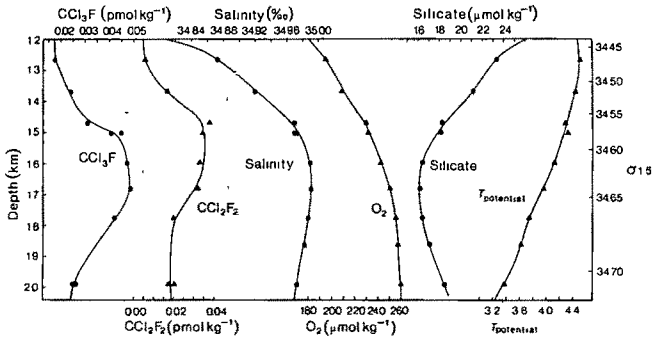


Fig. 3 Vertical profiles of CCl_3F, CCl_2F_2, salinity, oxygen, silicate and potential temperature at TAS Station 55 (0°03′ N, 44°10′ W) versus depth Corresponding values of the potential density anomaly $\sigma_{1.5}$ in kg m^{-3} are labelled along the right ordinate.

nounced maxima in CCl_3F and CCl_2F_2 concentrations at depths of 1.5–1.7 km, with the highest concentrations lying near the South American continent and with detectable maxima extending as much as 1,200 km seaward. The CCl_3F section in Fig. 2, which extends from Station 55 near the continental margin at the Equator to Station 64 near the Mid-Atlantic Ridge, shows an example of this pattern. The CFM concentrations in this mid-depth feature also decrease toward the south, with maximum CCl_3F values ranging from ~0.1 pmol kg^{-1} in the section at 13° N to ~0.015 pmol kg^{-1} in the section at 9° S. The CFM data thus suggest that this feature is produced by a southward-flowing western boundary undercurrent.

The relationship of this feature to the regional hydrography is shown in Fig. 3 by the depth profiles of CCl_3F, CCl_2F_2, salinity, O_2, silicate and potential temperature (the temperature a parcel of water would have if moved adiabatically to the sea surface) at TAS Station 55. The maxima in CCl_3F and CCl_2F_2 coincide with a maximum in salinity and a minimum in silicate which mark the core of the Upper North Atlantic Deep Water (UNADW)[11]. The potential density anomaly $\sigma_{1.5}$ (defined[12] by $\sigma_{1.5} = \rho_{1.5} - 10^3$, where $\rho_{1.5}$ is the density[13] expressed in kg m^{-3} that a parcel of seawater would have if moved adiabatically to the depth where the pressure is 1,500 dbar) of this layer is ~34.63 kg m^{-3}.

Tropical Atlantic waters in the potential density range of the UNADW are influenced by waters originating both in the Mediterranean Sea and in the region of the Labrador Sea. The salinity maximum of the UNADW is of Mediterranean origin, although in the western tropical Atlantic it has been truncated, and thus moved to a greater potential density, by the intrusion from the south of the overlying low-salinity Antarctic Intermediate Water (AAIW). Similarly, the silicate minimum, which is characteristic of waters of both Mediterranean and Labrador Sea origin, is truncated by the intrusion of overlying high-silicate AAIW. We emphasize that the coincidence of the depths of the CFM maxima with the extrema in salinity and silicate results simply from truncation of the profiles by low-CFM AAIW and does not necessarily indicate a common source region for these species in the UNADW.

The truncation of the UNADW extrema by the AAIW illustrates a major difficulty in using the core layer method to trace the waters in the UNADW to their source regions. We have instead evaluated the origin of the high CFMs in the UNADW by isopycnal layer analysis[12] using the $\sigma_{1.5} = 34.63$ kg m^{-3} potential density surface. This surface coincides with the UNADW CFM maxima we observe in the western tropical Atlantic and is characterized in this region by a potential temperature of ~4.0 °C and a salinity of ~34.98‰. Towards the north-east, the potential temperature and salinity on this surface increase in response to the influence of the Mediterranean outflow, reaching values of ~5.1 °C and ~35.20‰ at the northernmost TAS stations in the Canary Basin. Towards the north-west, our NAS data show decreases in potential temperature and salinity on this

isopycnal surface as the proportion of Mediterranean water is reduced. This effect is most pronounced at our northernmost stations near the Grand Banks, where this surface intersects a western boundary current of Labrador Sea Water (LSW) which is marked by decreases in potential temperature and salinity from ~3.6 °C and ~34.92‰ at Station 226 to ~3.3 °C and ~34.86‰ at Station 222. Throughout our study area, the depth of this isopycnal surface lies at ~1.6 km, except near the Grand Banks where it shoals to ~1.0 km.

The potential density and formation rate of LSW during 1948–75 have been evaluated[14] using its potential vorticity minimum as a tracer. Because that study used an earlier equation of state, its values of $\sigma_{1.5}$ must be reduced by 0.03 kg m^{-3} to place them on the same scale as ours. Thus, our $\sigma_{1.5}$ = 34.63 kg m^{-3} isopycnal surface lies near the lower limit of the LSW potential density range, ~0.03 kg m^{-3} less than the most common density of the LSW core. Based on temporal variations in salinity, the $\sigma_{1.5}$ = 34.63 kg m^{-3} surface in the Labrador Sea is believed[14] to have been actively renewed during 1948–63 and to have been relatively isolated from renewal subsequently during 1964–71.

The geographical distribution of CCl_3F concentrations on the $\sigma_{1.5}$ = 34.63 kg m^{-3} isopycnal surface at our TAS and NAS station locations is shown by the contours in Fig. 1. As an approximate compensation for the 1-yr difference in expedition dates, we have increased the values at the NAS stations by 6% to make the contours in this region approximately synoptic with the TAS measurements. In the equatorial region, these distributions do not show increasing CFM concentrations as one moves along the isopycnal surface from the western boundary of the Atlantic towards the high salinity water which extends outward from the Mediterranean. In the western Atlantic, however, CFM concentrations on the isopycnal surface increase steadily towards the Labrador Sea source region in the north. As well as these geographical relationships, we note that increasing CFM concentrations correlate with decreasing salinity everywhere on this isopycnal surface, except in the region of TAS Stations 79–82, where the influence of CFMs from the Mediterranean is clearly reflected by a correlation between increasing CFM concentrations and increasing salinity. We therefore conclude that the CFMs in the UNADW of the tropical western Atlantic must originate in the region of the Labrador Sea.

We have used a simple model to calculate the approximate time required for water to be transported from the surface of the Labrador Sea to the UNADW in the western tropical Atlantic, as well as the degree of dilution which occurs during this process. We have assumed that CCl_3F and CCl_2F_2 concentrations in Labrador Sea surface waters during wintertime convection are in approximate solubility equilibrium with the atmosphere, and that during their southward transport in a western boundary undercurrent they are diluted by mixing with surrounding waters which are essentially CFM-free. In this model, the transport time for the CFM-bearing component is given by the CCl_3F/CCl_2F_2 ratio, and the degree of dilution is given by the concentrations.

In these calculations, we have used the temporal variations in tropospheric CFM mixing ratios discussed above, and solubilities in LSW of 3.5 °C potential temperature and 34.9‰ salinity, expressed in terms of the F solubility function[15], of 0.0213 mol kg^{-1} atm^{-1} for CCl_3F and 0.00533 mol kg^{-1} atm^{-1} for CCl_2F_2 (M.J.W. and R.F.W., unpublished gas chromatographic solubility measurements).

The NAS measurements of LSW near the Grand Banks at ~47 °N and near Nova Scotia at ~42° N show CCl_3F/CCl_2F_2 ratios of 2.25 ± 0.1, which correspond to an equilibrium atmospheric ratio of 0.56 ± 0.02. This ratio is indistinguishable from the atmospheric ratio of 0.56 in 1981 during the NAS expedition, and because of the constancy of the CCl_3F/CCL_2F_2 ratio during the late 1970s, our ratio measurements can only constrain the mean transport time of these waters to <9 yr. Calculated dilution factors for the LSW found in this region are also poorly constrained, by the uncertainty in the transport time and by the range of the measured concentrations, to between zero and three-fold.

The TAS measurements of UNADW in the western boundary undercurrent of the equatorial Atlantic show CCl_3F/CCl_2F_2 ratios ranging from 1.2 to 1.5, with a mean value of ~1.4. Although we expect from the southward decrease in CFM concentrations that there is a corresponding decrease in CCl_3F/CCl_2F_2 ratio between 13° N and 9° S, we are unable to detect a significant trend because of the large error associated with the ratio measurement at these low concentrations. Including analytical errors, the mean atmospheric CCl_3F/CCl_2F_2 ratio in equilibrium with these waters is 0.35 ± 0.11, which corresponds to the atmospheric composition in 1960 ± 5 yr. The degree of dilution which this water has experienced is very strongly dependent on the uncertainty in the calculated transport time because the concentrations of CCl_3F and CCl_2F_2 in the atmosphere increased approximately seven-fold and four-fold respectively, between 1955 and 1965. For surface water equilibrated with the atmosphere in 1960, we calculate that the CCl_3F concentration of 0.05 pmol kg^{-1} found near the South American continent at the Equator has been diluted approximately five-fold by CFM-free water.

Thus, our data show that waters originating in the region of the Labrador Sea have transported atmospheric CFMs into the equatorial Atlantic along a western boundary undercurrent of UNADW, a distance of about 10,000 km, in about 23 yr. This corresponds to a mean velocity of about 1.4 cm s^{-1}. In the equatorial region, the CFM distributions show that this western boundary flow crosses into the Southern Hemisphere to at least 9° S and suggest that the flow also extends eastward along the Equator.

It is important to recognize that the transport times and dilution factors we have calculated are strongly model dependent. Because atmospheric CFM concentrations have tended to rise exponentially rather than linearly, if subsurface mixing occurs between CFM-bearing waters of different ages, the resulting apparent age will be biased in favour of the younger component. Also, the assumption of atmospheric equilibrium in surface waters may be inappropriate in convective processes and under such conditions the CFM ratios may be affected by kinetics. Further research in the Labrador Sea and in other areas of active water-mass formation is essential for quantifying these relationships.

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1 *Halocarbons Effects on Stratospheric Ozone* (National Academy of Sciences, Washington, DC, 1976)
2 Cunnold, D M *et al J geophys Res* **88**, 8379–8400 (1983)
3 Cunnold, D M *et al J geophys Res* **88**, 8401–8414 (1983)
4 Broecker, W S & Peng, T-H *Tellus* **26**, 21–35 (1974)
5 Gammon, R. H, Cline, J & Wisegarver, D *J geophys Res* **87**, 9441–9454 (1982)
6 Bullister, J L & Weiss, R F *Science* **221**, 265–268 (1983)
7 Rasmussen, R A & Lovelock, J E *J geophys Res* **88**, 8369–8378 (1983)
8 Rasmussen, R A & Khalil, M A K in *Proc. NATO Advanced Study Inst Atmospheric Ozone* (ed Aiken, A C) 209–231 (Federal Aviation Administration, Washington, DC, 1980)
9 *Production, Sales and Calculated Release of Chlorofluorocarbons 11 and 12 through 1982* (Chemical Manufacturers Association, Washington, DC, 1983)
10 Molina, M J. & Rowland, F S *Nature* **249**, 810–812 (1974).
11 Wust, G *Wiss Ergebn dt atlant Exped 'Meteor'* **6**, 109–288 (1935)
12 Lynn, R J & Reid, J L *Deep-Sea Res* **15**, 577–598 (1968)
13 Millero, F J, Chen, C-T, Bradshaw, A & Schleicher, K *Deep-Sea Res* **27A**, 255–264 (1980)
14 Talley, L D & McCartney, M S *J phys Oceanogr* **12**, 1189–1205 (1982)
15 Weiss, R F & Price, B A *Mar Chem* **8**, 347–359 (1980)

Sulphate and nitrate concentrations in snow from South Greenland 1895–1978

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An understanding of the phenomenon of acid rain requires the identification of the sources of the species affecting the *p*H of rainwater, both natural and anthropogenic, and their temporal and spatial development. The scant data concerning the historical development of the acidity in precipitation are from urban regions or their vicinity, where local effects dominate and obscure the hemispherical pattern[1]. The Greenland ice sheet allows us to trace the evolution of the acid rain in a remote location that is free from local effects. Sulphuric and nitric acids are the two species that dominate the acidity in precipitation[2–4]. We report here measurements of $[SO_4^{2-}]$ and $[NO_3^-]$ in firn samples spanning the period 1895–1978. Samples, each covering 1 yr, were taken from a 70-m core drilled at Dye 3, South Greenland; $[NO_3^-]$ and $[SO_4^{2-}]$ both increased by a factor of ~2 during the period. By comparing the recent concentrations of nitrate and sulphate with those resulting from natural sources, we conclude that anthropogenic emissions of the precursors (NO_x, SO_2) had already surpassed natural sources in the late 1950s.

In 1980 a shallow electromechanically drilled core was collected near Dye 3 (65°11'N, 43°30'W) in the frame of the US–Danish–Swiss Greenland Ice Sheet Program. The main purpose of this core was to provide samples for studying the influence of the 11-yr solar cycle on the ^{10}Be concentration in the firn[5]. As a supplementary study, anion concentrations were determined by ion chromatography as possible correction factors to eliminate 'meteorological noise' in the ^{10}Be data. To avoid contamination, the drill site was located 2 km north of the radar station and perpendicular to the main wind direction. In the field, the acidity was qualitatively determined by solid electrical conductivity measurement (ECM)[6] and a small strip of the core was cut for continuous $\delta^{18}O$ analysis with a resolution of eight samples per calculated year. After field sampling, the cores were packed in polyethylene tubes and sent back to the laboratory. The volcanic Katmai eruption, which took place in 1912 in Alaska, is clearly visible in the ECM record and served as an internal check on the accuracy of the timescale used. The $\delta^{18}O$ and ECM results showed the mean snow accumulation rate at Dye 3 to be 50-cm water equivalent yr^{-1} and provided an age/depth relation which allowed us to cut the core into annual samples. To remove external contamination before chemical analysis, the outer layer of the firn was removed with a stainless steel microtome knife. All sample treatment was done in a cold room (−20 °C). The samples were melted in a covered polyethylene beaker and aliquots were taken for chemical analysis. To check whether the sampling and melting procedure introduces any noticeable background, we treated ultra-pure water in the same manner as the samples. This water was frozen in the polyethylene beaker in the cold room and re-melted in the laboratory. Blank values were below 8 parts per 10^9 (p.p.b.) (ng per g ice) for the measured species; the reproducibility of the measurements was better than 5%. Figures 1 and 2 show $[NO_3^-]$ and $[SO_4^{2-}]$ during the period 1895–1978. To reveal the general trend, a spline-smoothed curve has been drawn through the points. Nitrate and sulphate show an increasing trend with a large year-to-year fluctuation; our measured concentrations can be compared with published data[7–9]. For the years 1895–1904 we measured a mean sulphate level of 36 p.p.b., which is higher than for the two time periods 1539–1577 and 1773–1791, where the sulphate level was determined by Herron[7] to be 22 p.p.b.

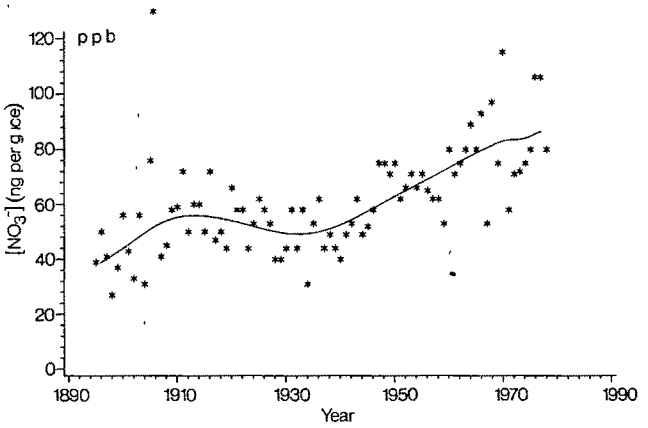


Fig. 1 Annual $[NO_3^-]$ in a shallow core from Dye 3, South Greenland. The general trend is shown with a spline-smoothed curve.

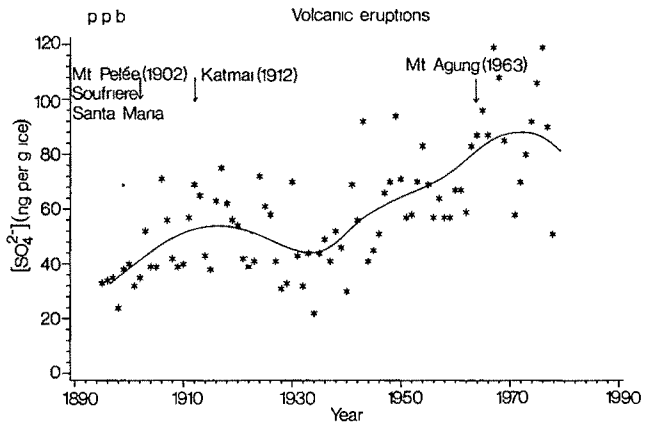


Fig. 2 Annual $[SO_4^{2-}]$ in a shallow core from Dye 3, South Greenland. The general trend is shown with a spline-smoothed curve.

Under unfavourable meteorological conditions the radar station is a possible source of contamination, especially for sulphate. However, the measured $[SO_4^{2-}]$ of 90 p.p.b. for the recent time period 1973–1978 agrees well with Herron's level of 85 p.p.b. for the time period 1975–1981, which was determined in a pit study much further from the Dye 3 station than was our drilling site. Furthermore, our sulphate and nitrate data do not show a discontinuity over the year 1961, when the radar station was constructed. We conclude, therefore, that the contamination attributable to emissions from the radar station is small. We found no indication that the occurrence of melt layers in the Dye 3 firn core influences $[NO_3^-]$ and $[SO_4^{2-}]$.

The nitrate levels measured by Herron[7] are generally slightly higher than ours (Herron's 1884–91, 51 p.p.b., our 1895–1904, 44 p.p.b.; Herron's 1975–81, 114 p.p.b., our 1973–78, 86 p.p.b.). The high nitrate value we found for 1906 is surprising; we cannot exclude contamination of this sample, particularly as $[SO_4^{2-}]$ is also relatively high and in Rasmussen's Dye 3 data[8], $[NO_3^-]$ for 1906 is not elevated. As a consequence of the 1908 Tunguska impact in Siberia, Turco *et al.*[10] have calculated that NO_x concentrations in the lower stratosphere would be elevated by an order of magnitude for a period over 1 yr. The years 1908–10 do not, however, show a higher $[NO_3^-]$, so that the predicted enhancement of $[NO_x]$, if it occurred, did not penetrate into the troposphere. Similar conclusions were reported by Rasmussen *et al.*[8], based on their measurements at Dye 3 and Camp Century.

The concentration depends on three factors: the scavenging process, the transport of air masses from the source to the receptor and the source strength. We ascribe the general trend in anion concentrations to increasing source strength and the short-time fluctuations to the variability in transport processes and scavenging.

Although NO_3^- and SO_4^{2-} are mainly incorporated as HNO_3

and H_2SO_4 in the ice[11-13], HNO_3 and H_2SO_4 are not emitted directly into the atmosphere. The precursors for HNO_3 are NO and NO_2, and for H_2SO_4 are SO_2, dimethyl sulphide (DMS) and H_2S. All these species are oxidized in the atmosphere, either in the gas phase or in cloud droplets[14] to form the two acids, which are then effectively removed by precipitation. The global sources or precursors of HNO_3 and H_2SO_4 are compiled in Table 1. The range of estimates in the literature is large, especially for the natural sources, which cannot be derived from economic data as can the anthropogenic sources. The volcanic contribution is, of course, subject to extreme fluctuation. During the time period covered by our samples, the volcanic activity was low except for the years 1902, 1912 and 1963[15]. With the exception of the already mentioned Katmai explosion, all large eruptions were in the equatorial zone. Their contribution to the sulphate level in our samples is not distinguishable from the scatter.

Inputs from land-based sources to the impurity level in Greenland must travel quite far and must be able to survive in the atmospheric boundary layer, where removal by precipitation is very effective. Sources in the free troposphere or in the ocean surrounding Greenland will contribute more easily to the concentration in the precipitation of South Greenland. Essentially all anthropogenic sources are land-based with the exception of jet airplanes.

Using the estimated source strengths of the different NO_3^- and SO_4^{2-} precursors, one can calculate the percentage of the '1900 level' of $[NO_3^-]$ and $[SO_4^{2-}]$ that is derived from land-based sources. Our calculation assumes that the different sources contribute linearly to the impurity level in the snow and are split into continental (land-based) and other (non-land-based) sources. No interhemispherical mixing is assumed. Two time periods, 1895–1901 and 1973–78, were considered, assuming the emission rates indicated in Table 1 and using the mean measured concentration of these time intervals.

The '1900 level' may be expressed as $\gamma P_C + \delta P_O = C_{1900}$; the 'recent level' as $\gamma(P_C + P_A) + \delta P_O = C_{recent}$, where γ and δ are factors linking emission rates and firn concentration; P_C, P_A and P_O are the land-based (continental), anthropogenic (~1975) and non-land-based (ocean) production rates, respectively; and C_{1900} and C_{recent} are the '1900' and 'recent' firn concentrations, respectively.

This model is no more than a first approximation. Natural sources are diffuse, whereas the anthropogenic components have more the character of point sources. The results of this model calculation give for the 1900 level a contribution of land-based sources to the concentration in the ice of 54% for sulphate and 37% for nitrate. It should be pointed out that this number depends strongly on the emission rates that we have selected in Table 1.

Table 1 Global emission ranges

Sulphur source		Northern Hemisphere range (Tg S yr^{-1})	Southern Hemisphere range (Tg S yr^{-1})
Natural·	Biogenic sea	14–32	18–40
	Biogenic land	2–74 (32)*	1–36
	Volcanoes	0 6–6 (3)*	0 4–4
Man-made:	(1976)	69–98 (98)*	5–6
Nitrogen source			
Natural:	Soil	0.7–7 (4)†	0.3–3
	Wildfire	0.2–0.6 (0 4)†	0.1–0.3
	Ox. NH_3	0–3 (1.9)†	0–2
	Biomass burn.	3–8 (5.5)†	
	Lightning	1–4	1–4
	Stratosphere	0.15–0 45	0.1–0 45
Man-made:	Fossil fuel	7–17 (13)†	0.5–1
	Biomass burn.	6–40 (18)†	6–18
	Jets	0.2–0.4 (0 3)†	<0.1

Data compiled from refs 19–24.

* Assumed production for calculations. natural production land-based, 35 Tg yr^{-1} (≈total land-based production pre-industrial), natural and man-made production land-based, 133 Tg yr^{-1}.

† Assumed production for calculations: natural production land-based, 11.4 Tg yr^{-1} (≈total land-based production pre-industrial), natural and man-made production land-based, 43 Tg yr^{-1}

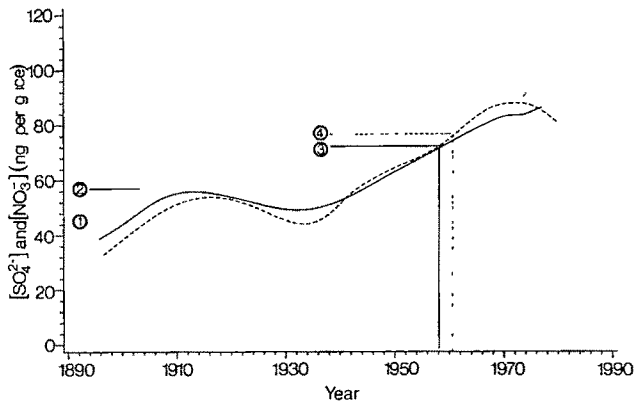


Fig. 3 Time development of atmospheric load with nitrate(—) and sulphate (- - - -) Comparison of anthropogenic and natural input. (1) '1900' $[SO_4^{2-}]$ $(+1\sigma)$ (2) '1900' $[NO_3^-]$ $(+1\sigma)$. (3) '1900' $[NO_3^-]$ $(+1\sigma)+16$ p.p.b. (≈11 Tg N yr^{-1} anthropogenic emission) for when anthropogenic N sources were approximately equal to global natural N sources. (4) '1900' $[SO_4^{2-}]$ $(+1\sigma)+28$ p.p.b. (≈57 Tg S yr^{-1} anthropogenic emission) for when anthropogenic S sources were approximately equal to global natural S sources.

Based on our calculation, 1 Tg yr^{-1} of land-based sulphur source corresponds in the mean annual concentration of the snow in Dye 3 to an additional $[SO_4^{2-}]$ of ~0.6 p.p.b., whereas 1 Tg yr^{-1} of land-based nitrogen source corresponds to ~1.4 p.p.b. of additional $[NO_3^-]$, respectively. These numbers depend only weakly on the assumed emission rates. Thus, the source distribution and the long-range transport of the sulphur and nitrogen components seem to be different. Our data suggest that the nitrogen component is more easily transported through the boundary layer than is the sulphur component, which seems to be contrary to the calculation published by Rhode *et al.*[16]. However, their calculation was based on precipitation data from the European Air Chemistry Network, whereas our data apply to Greenland. The low proportion of the land-based contribution to the pre-industrial $[NO_3^-]$ supports the idea that NO_x from the stratosphere and produced by lightning are the main sources of the background nitrate in remote regions[6,17]. Such sources in the free troposphere were also postulated by Huebert and Lazrus[18] to explain their $[HNO_3]$ measurements in the free troposphere.

Our sulphate concentration in the period 1895–1904 is higher than the values given by Herron[7] for earlier periods. Because volcanic activity was low during 1895–1904, our higher measured concentration indicates that by this time the sulphate level was already considerably elevated over pre-industrial values, possibly reflecting early industrial activity. Our model is inconsistent with the pre-industrial sulphate level given by Herron (22 p.p.b.) and would require a land contribution of over 100%. The most probable explanation is that the natural land-based source used in our model is an overestimate.

Using the relation given above between concentration increase in the ice and source strength, we can deduce the time when anthropogenic sources surpassed the natural source concentration given in Table 1. Figure 3, illustrating this behaviour, shows that the anthropogenic sources started to dominate the total natural sources around 1960. This agrees with the anthropogenic emission data for sulphate given by Möller[19].

Our data suggest that man-made pollution had already started to affect remote regions more than 30 yr ago. The nitrate and sulphate concentrations in Greenland contain information which can ultimately be used to interpret the development and

distribution of sources of acid rain, although at the moment our understanding of source strengths and transport is still so rudimentary that the interpretation must remain largely qualitative. More widespread drilling of firn cores in polar regions would allow the historical development of the acid deposition pattern to be established and sophisticated source receptor models to be tested. Nevertheless, the data already show that human activity drastically changed the atmospheric load of the acidic trace species HNO_3 and H_2SO_4. This fact emerges more clearly in the historical record from remote locations, such as Dye 3, than from direct measurements in populated areas which are influenced by changes in measuring technique and changed local distribution of pollution with time.

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1. Hidy, G. M. *et al. Air Pollution Control Ass. J.* **31**, 333-354 (1984).
2. Likens, G. E. *Scient. Am.* **241** (4), 43-51 (1979).
3. Marsh, A. R. W. *Atmos. Envir.* **12**, 401-406 (1978).
4. Charlson, R. J. & Rhode, H. *Nature* **295**, 683-685 (1982).
5. Beer, J. *et al. Radiocarbon* **25**, 269-278 (1983).
6. Neftel, A. *et al. Greenland Ice Sheet Program*, Philadelphia (Am. geophys. Un. Monogr, 1984).
7. Herron, M. M. *J. geophys. Res.* **87**, 3052-3060 (1982).
8. Rasmussen, K. *et al. Icarus* **58**, 101-108 (1984).
9. Herron, M. M. *Am. chem. Soc. Symp. Ser.* **176**, 303-318 (1982).
10. Turco, R. P. *et al. Science* **214**, 19-23 (1981).
11. Legrand, M. & Delmas, R. J. *Atmos. Envir.* **18** (9), 1867-1874 (1984).
12. Hammer, C. U. *J. phys. Chem.* **87**, 4099-4103 (1983).
13. Delmas, R. J. *et al. J. geophys. Res.* **87**, 4314-4318 (1982).
14. Chameides, W. L. & Davis, D. *Chem. Engng News* **60**, 38-52 (1982).
15. Lamb, H. H. *Phil. Trans. R. Soc.* **A266**, 425-533 (1970).
16. Rhode, H. *et al. Tellus* **33**, 132-141 (1981).
17. Hameed, S. *et al. Geophys. Res. Lett.* **8**, 591-594 (1981).
18. Huebert, B. J. & Lazrus, A. L. *J. geophys. Res.* **85**, 7322-7328 (1980).
19. Möller, D. *Atmos. Envir.* **18**, 29-36 (1984).
20. Hahn, J. & Crutzen, P. J. *Phil. Trans. R. Soc.* **B296**, 521-541 (1982).
21. Ehhalt, D. H. & Drummond, J. W. in *Chemistry of Unpolluted and Polluted Troposphere* (eds Georgii, H. W. & Jaeschke, W.) 119-151 (Reidel, Dordrecht, 1982).
22. Cullis, C. F. & Hirschler, M. M. *Atmos. Envir.* **14**, 1263-1278 (1980).
23. Möller, D. *Atmos. Envir.* **18**, 19-27 (1984).
24. Nguyen, B. C. *et al. J. geophys. Res.* **88**, 10903-10914 (1983).

Late Eocene microtektites and radiolarian extinctions on Barbados

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A late Eocene microtektite layer has been found in three exposed sections on Barbados, West Indies. These microtektites and associated tektite fragments are compositionally similar to the North American tektites and thus, like the Caribbean and Gulf of Mexico microtektites, may belong to the strewn field of 'North American' microtektites which have been found in deep-sea deposits from the Caribbean Sea, across the Pacific and into the northeastern Indian Ocean[1,2]. The 'North American' layer has been reported to be approximately synchronous with several radiolarian extinctions[3,4] and with an anomaly in iridium concentrations[5-7], and it has been hypothesized that the microtektites, the extinctions and the iridium anomaly are all related to an asteroid impact. We report here that the radiolarian extinctions predate the microtektite layer and that the highest Ir concentrations apparently coincide with the extinctions. Thus, although the extinctions may be causally related to the Ir anomaly, the microtektites appear not to be.

Table 1 Major oxide composition of selected Barbados microtektites and tektite fragments and North American tektites

Sample no.	SiO_2	Al_2O_3	FeO	MgO	CaO	Na_2O	K_2O	TiO_2
Barbados microtektites								
179-16-2	84.2	10.4	1.48	0.45	0.20	0.60	1.77	0.49
176-16-14	79.4	11.7	3.23	0.61	0.35	1.28	2.54	0.54
173F	78.6	13.0	3.15	0.99	0.63	0.73	1.88	0.61
173E	77.5	12.3	3.67	0.90	0.86	0.83	2.86	0.77
179-16-5	76.0	13.5	3.68	0.96	0.78	1.23	2.78	0.64
Barbados tektite fragments								
173G	81.7	10.2	2.06	0.41	0.94	1.63	2.53	0.43
173H	81.0	10.3	2.71	0.49	0.73	1.56	2.56	0.43
181A	78.9	12.4	3.28	0.56	0.17	1.41	2.04	0.81
173C	78.8	12.6	3.20	0.54	0.46	1.34	2.09	0.60
173D	78.6	12.8	3.18	0.52	0.50	1.31	2.04	0.70
North American tektites								
BM	81.31	10.96	2.43	0.53	0.50	1.50	2.17	0.53
181D	81.0	11.0	2.58	0.60	0.36	0.84	2.61	0.52
181C	78.4	12.5	3.22	0.62	0.41	1.42	2.25	0.66
B-50	78.19	12.35	3.50	0.73	0.65	1.71	2.23	0.74
B-6	76.25	13.49	3.98	0.74	0.74	1.63	2.22	0.74

The data for North American tektite samples BM, B-50 and B-6 are from ref. 14. The rest of the data were obtained by energy dispersive X-ray analysis. A total of 18 microtektites and five tektite fragments from Bath Cliff were analysed.

Microtektites belonging to two Quaternary tektite-strewn fields on land, the Australasian and Ivory Coast, have been found in deep-sea sediment cores taken in the Australasian/Indonesian region and off the Ivory Coast, respectively[1]. Much older than the Quaternary group are the late-Eocene North American microtektites, found in a low-latitude band stretching from the Caribbean to the northeastern Indian Ocean. The glassy rounded tektites and microtektites are believed to be produced by impacts[8,9]. Correlation of late-Eocene microtektites, anomalous Ir, and radiolarian extinctions[6,7] has been used to suggest a relationship between impacts and extinctions similar to that which has been inferred for the Cretaceous-Tertiary event[10].

Recent detailed biostratigraphical investigations on Barbados[11] provided the possibility of locating, on land, the stratigraphical level of occurrence of the microtektites, which had previously been available in only very limited amounts, at scattered localities, in deep-sea cores. The stratigraphical resolution attainable in these land-based sections is 2-10 times greater than that attained in deep-sea sediment cores containing the microtektite layer. For example, the stratigraphical interval separating the earliest occurrence of the radiolarian *Thyrsocyrtis bromia* and the evolutionary transition of *Lithocyclia aristotelis* to *L. angusta* is 100-110 m at Bath Cliff, 10-20 m at Deep Sea Drilling Project Site 149 (ref. 12), about 10 m at DSDP Site 543 (unpublished data) and 15-45 m at DSDP Site 94 (ref. 13). The microtektites were found in this interval at a single stratigraphical level (10-30 cm thick) in three sections; Bath Cliff, Gay's Cove North and Gay's Cove South.

All three sections were sampled continuously, by taking contiguous samples 5 cm thick. This provided a basis for examining changes in the microtektites throughout their occurrence, and details of the abundances in the uppermost parts of the ranges of radiolarian species as they became extinct. Weighed subsamples of the channel samples were disaggregated and sieved to obtain the >63-μm fraction for investigation of the radiolarians and the microtektites.

Identification of the Barbadian microtektites and tektite fragments as belonging to the late-Eocene horizon which has been called the 'North American' strewn field is indicated by their late Eocene age and by their chemical composition (Table 1). North American tektites are characterized by low lime and magnesia content as compared with tektites from other strewn fields[14], and this is reflected in the composition of the Barbados microtektites and tektite fragments. They have a unimodal size distribution, this being more pronounced in the samples near

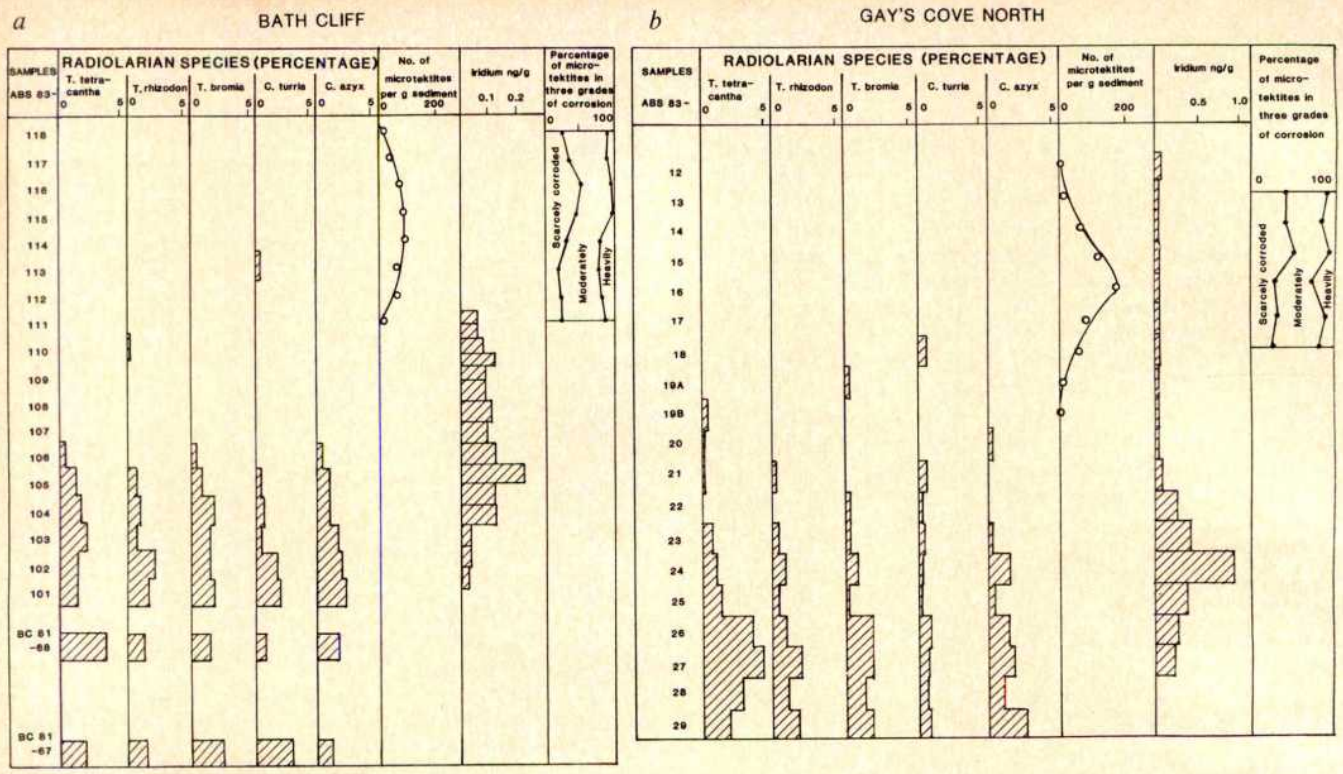


Fig. 1 The upper limits, in the Bath Cliff (*a*) and Gay's Cove North (*b*) sections, of species that became extinct slightly below the microtektite layer. Abundances are given as percentages of the total assemblage coarser than 63 μm, estimated from assemblages of 6,000–26,000 radiolarians from each sample. Although the five radiolarian species plotted constitute a high proportion of the 20 or so commonly used for late-Eocene/early-Oligocene stratigraphy, the proportion is less remarkable when considered in relation to the 200–300 additional species present in these assemblages but not used stratigraphically. In the right-hand columns are numbers of microtektites and their degree of corrosion, and Ir concentrations. In each section, the extinctions clearly predate the microtektite event, and coincide with the high Ir concentrations.

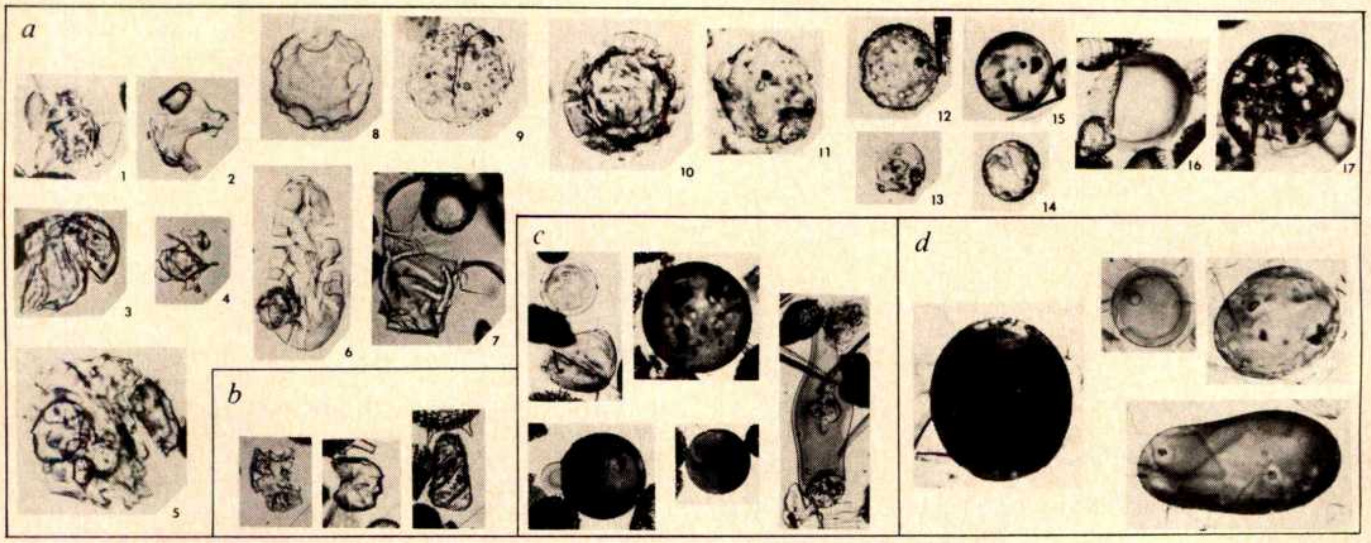


Fig. 2 Representative examples of microtektites from: *a*, Barbados; *b*, DSDP Site 543; *c*, DSDP Site 149; and *d*, DSDP Site 94. In *a*, specimens 15–17 were counted as smooth, regular; specimens 8–14 as moderate; and 1–7 as highly irregular. Magnification ×105.

the middle of the layer than towards its limits. The 80–100-μm and 100–120-μm size grades contain more specimens than do the other 20-μm increments, but the greatest volume of material is in the slightly larger size grades. Assuming a grain bulk density of 2.5 g cm^{-3} for both the sediment and the tektite material and an average bulk dry density of 1.32 g cm^{-3} for the sediment[11], the net fluence of microtektites and tektite fragments is 45 tonnes km^{-2} at the Bath Cliff section. Although the thicknesses of the layer at Gay's Cove North and Gay's Cove South is less certain because of imprecise relations between the line of sampling and the attitude of the beds (resulting from local small-scale tectonic complications), the total tektite contribution appears to be greater, perhaps as much as 80 tonnes km^{-2} in the former section and 100 tonnes km^{-2} at the latter.

This is substantially less than the 1,000 tonnes km^{-2} calculated for Caribbean and Gulf of Mexico sites[3]. The lower figures for Barbados may indicate that this site is closer to the edge of the strewn field. Another factor is that the Barbados microtektites are now above sea level and have probably experienced more solution than the microtektites from the submarine localities.

In an attempt to detect the iridium anomaly reported just below the main microtektite layer by Ganapathy[6], and coincident with it by Alvarez *et al.*[7], neutron activation analysis was performed on a continuous series of Bath Cliff samples from about 5 cm above the base of the layer to 25 cm stratigraphically below it, and on a continuous series of Gay's Cove North samples from about 15 cm stratigraphically above the microtektite peak to about 30 cm below it. The results show a peak in Ir concentration well below the microtektite layer and at practically the same level as the radiolarian extinctions in both sections (Fig. 1*a*, *b*), but it represents a substantially lower net fluence of Ir (<2 ng cm^{-2} at Bath Cliff and ~ 7 ng cm^{-2} at Gay's Cove North) than would be expected on the basis of results obtained by Ganapathy[6] (63 ng cm^{-2}). Our data, like Ganapathy's, would indicate that the anomalous Ir is not coincident with the North American microtektite layer, but we cannot rule out a relationship between the radiolarian extinctions and another impact event. Clinopyroxene-bearing glass spherules (as distinct from the microtektites) are coincident with the Ir anomaly in core RC9-58 (refs 6, 15). These may have once existed at the level at which we found the Ir, and have since been dissolved by ground water above sea level.

The radiolarian extinctions clearly predate the microtektite layer in these sections (Fig. 1). Their apparent synchroneity in sections investigated earlier may be due to much lower rates of sediment accumulation and thus lower stratigraphical resolution. The tailing-upward of the radiolarian species could be attributable to bioturbation[16] (and the times of their extinction pinpointed at slightly above the levels at which their abundances begin markedly to decline). However, bioturbation does not provide an explanation for the separation between the microtektites and the extinctions. At Bath Cliff, the separation of ~ 27 cm probably represents an interval of 13,000–14,000 yr. This is based on an estimated sediment accumulation rate of ~ 20 m Myr^{-1} (calculated from separation of biostratigraphical events)[11,17]. This contradicts any causal relationship between the extinctions and the late-Eocene microtektite event[6,7].

Although compositional similarities and stratigraphical position imply a correlation between the Barbadian microtektites and the North American field, there are differences that may reflect variations within the field. Texturally, these samples range from regular spheres and teardrops to highly corroded or angular fragments. The proportion of fragments appears to be much greater in Barbados and at DSDP Site 543 (generally $>60\%$, Fig. 2*a*, *b*) than in the Caribbean and Gulf of Mexico sites ($<8\%$ (ref. 4); Fig. 2*c*, *d*). Moreover, the Barbadian microtektites have a slightly higher SiO_2 content and have a generally paler colour than those from the Caribbean and Gulf of Mexico. Keller *et al.*[18] have suggested that there may be multiple late-Eocene microtektite layers, not associated with profound changes in the biota, but this cannot be evaluated here. The basic question of the multiple falls will ultimately be resolved by careful description of the physical, chemical and isotope characteristics of the microtektites at each of their occurrences and by their placement in a more precise biostratigraphical framework[19,20].

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1. Glass, B. P., Swincki, M. B. & Zwart, P. A. *Proc. 10th Lunar planet. Sci. Conf.* 2535–2545 (1979).
2. Glass, B. P. & Crosbie, J. R. *Am. Ass. petrol. Geol. Bull.* **66**, 471–476 (1982).
3. Maurrasse, F. & Glass, B. *Trans. VII Conf. Géol. Caraïbes* 205–212 (1976).
4. Glass, B. P. & Zwart, M. J. *Bull. geol. Soc. Am.* **90**, 595–602 (1979).
5. Asaro, F., Alvarez, L. W., Alvarez, W. & Michel, H. V. *Geol. Soc. Am. Spec. Pap.* No. 190, 517–528 (1982).
6. Ganapathy, R. *Science* **216**, 885–886 (1982).
7. Alvarez, W., Asaro, F., Michel, H. V. & Alvarez, L. W. *Science* **216**, 886–888 (1982).
8. Barnes, V. E. & Barnes, M. A. *Tektites* (Dowden, Hutchinson and Ross, Stroudsburg, 1973).
9. Shaw, H. F. & Wasserburg, G. J. *Earth planet. Sci. Lett.* **60**, 155–177 (1982).
10. Alvarez, L. W., Alvarez, W., Asaro, F. & Michel, H. V. *Science* **208**, 1095–1108 (1980).
11. Saunders, J. B. *et al. Micropaleontology* **30**, 390–425 (1985).
12. Riedel, W. R. & Sanfilippo, A. in *Init. Rep. DSDP* **15**, 705–751 (1973).
13. Sanfilippo, A. & Riedel, W. R. *Init. Rep. DSDP* **10**, 475–611 (1973).
14. Chao, E. C. T. in *Tektites* (ed. O'Keefe, J. A.) 51–94 (University of Chicago Press, 1963).
15. Glass, B. P., DuBois, D. L. & Ganapathy, R. *Proc. 13th Lunar planet. Sci. Conf.* **1**, A425–A428 (1982).
16. Ruddiman, W. F. *et al. Sedim. Geol.* **25**, 257–276 (1980).
17. Berggren, W. A., Kent, D. V. & Flynn, J. J. *Geol. Soc. Lond. Spec. Pap.* (in the press).
18. Keller, G., D'Hondt, S. & Vallier, T. L. *Science* **221**, 150–152 (1983).
19. Glass, B. P. *Science* **224**, 309 (1984).
20. Keller, G., D'Hondt, S. & Vallier, T. L. *Science* **224**, 309–310 (1984).

Manipulation of the morphogenetic pathways of tobacco explants by oligosaccharins

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Acquiring the ability to regulate morphogenesis in plants has long been an elusive goal. Previous research has established that 'thin cell-layer' explants from the surface of the floral branches of tobacco can be induced to form either callus, vegetative buds, flowers or roots by adjusting the *p*H and the ratio of auxin to cytokinin in the culture medium[1–5]. We now report that oligosaccharins (oligosaccharides with regulatory activity) derived from plant cell walls can, at concentrations of $\sim 10^{-8}$ or 10^{-9} M, induce tobacco explants to form vegetative buds instead of flowers or callus, or flowers instead of vegetative buds. Other oligosaccharins can induce the explants to form roots instead of vegetative buds. This ability of oligosaccharins to control morphogenesis suggests that these fragments are *in situ* regulators of morphogenesis.

Tissue cultures of some plant species have been induced to form vegetative buds and/or roots by varying the relative concentration of growth regulators (for example, auxin and cytokinin)[1]. Despite extensive trials with various concentrations of growth regulators, carbohydrates and mineral salts, however, it remains difficult to induce tissue cultures of many plant species to form organs. Tissue cultures of those plant species that can be induced by means of varying the ouxin/cytokinin ratio usually form only vegetative buds, roots, or undifferentiated callus. Besides altering the concentration of these two growth regulators, adding sucrose or glucose[2,3] and varying the *p*H of the culture medium[4,5] are two other factors that have been shown to affect organ formation in tobacco tissue culture. Moreover, cultured tissues with organized cells (for example, epidermis, subepidermis) may have greatly increased capacities to form organs[6–10]. The ability to control morphogenesis in tissues has been particularly well developed in 'thin cell-layer' explants of tobacco[9,11,12]. We have used this system for our experiments.

The tobacco explants are obtained by excising cortical tissue of floral branches in 1×10-mm strips containing epidermis (1 cell layer), subepidermis (1 cell layer) and parenchyma (1–3 cell layers). When these thin cell-layer strips, or 'tobacco explants' as we will call them, are floated on liquid culture media containing salts, glucose (as an energy source), auxin (indolebutyric acid) and cytokinin (kinetin), and when the *p*H of the culture medium is adjusted to a specific value, the explants are induced to form flowers, vegetative buds, roots or undifferentiated callus. Thus, exogenously applied factors determine the specific type of organ formed by the explants.

Table 1 Effect of EPG fragments on morphogenesis of tobacco explants

	Concentration of EPG fragments ($\mu g\ ml^{-1}$)	Flowers	Vegetative buds	Callus formation
Medium I	0	600*	0	0
*p*H 3.8	1	100	1,800	0
Medium II	0	0	0	100%
*p*H 5.0	1	0	400	0
	10	0	1,200	0
*p*H 6.0	0	0	1,800	0
	1	600	18	0

* Total number of organs (flowers, floral shoots, vegetative buds or roots) formed or percentage of explants exhibiting callus formation (cell proliferation without organogenesis) in three separate experiments. The numbers are the sums in each experiment of the organs on 20 explants.

Methods. Tobacco plants were cultured at 24 °C under a 16-h photoperiod of natural light, complemented by artificial light (25 $W\ m^{-2}$) when the natural light was $< 50\ W\ m^{-2}$. The artificial light was a mixture of fluorescent (Mazda-type 65 W) and incandescent (60 W). Relative humidity was maintained at 65%. The tobacco explants were excised from basal parts of floral branches of *Nicotiana tabacum* L. c.v. samsun when the inflorescence contained 20 green fruits. The terminal green fruit of the cyme was 2.0–2.3 cm long and ~1.2 cm in diameter. The explants were obtained from floral branches that had been surface-sterilized (7% $Ca(OCl)_2$ for 10 min) and then rinsed three times in sterile water. Twenty explants were floated on liquid medium (40 ml) in Petri dishes (10 cm). The dishes were sealed with Parafilm. The basal medium was composed of Murashige and Skoog's macro- and micro-elements[29], *myo*-inositol (100 mg l^{-1}), thiamine-HCl (0.4 mg l^{-1}) and glucose (30 g l^{-1}). Kinetin was added at 5×10^{-7} M and indolebutyric acid (IBA) at 5×10^{-7} M in medium I and at 3×10^{-6} M in medium II. Each medium was adjusted to the specified *p*H with either HCl or KOH. Oligosaccharin mixtures (1 or 10 $\mu g\ ml^{-1}$) were added (the addition of the oligosaccharins did not alter the *p*H of the media). Each medium was sterilized by passing it through a Millipore filter (0.2 μm). The explants were incubated at 24 °C and under continuous light (20 $W\ m^{-2}$). After 30 days, the morphogenesis of the explants was assessed. Each experiment was repeated three or four times. The results presented here are a summary of the data collected in all of these experiments. Two different mixtures of sycamore cell wall fragments (oligosaccharins) were used. One set of oligosaccharins (EPG fragments) was released by endo-α-1,4-polygalacturonase treatment of isolated sycamore cell walls. Endopolygalacturonase was purified as described elsewhere[30], as were the cell walls[31]. The cell walls were treated with the enzyme and the solubilized fragments were isolated as described elsewhere[32], except that the *p*H of the fragments was adjusted to 6.5 before the final exhaustive dialysis against deionized water. The second mixture of sycamore cell wall fragments was prepared by partial base-catalysed solubilization and partial degradation of cell wall components. These B fragments were prepared as follows: 4 MKOH (500 ml), containing $NaBH_4$ (500 mg) was added to isolated sycamore cell walls (5 g). The suspension was stirred overnight and then passed through two layers of Whatman GF/A filter paper. While being vigorously stirred, the soluble material was adjusted to *p*H 6.5 with concentrated HCl. The solvent was evaporated at 40 °C under reduced pressure. The supernatant solution was decanted repeatedly from salt crystals that formed in the evaporation flask. The salt crystals were washed with a small volume of distilled water and the washes were added to the supernatant. Once the volume of the supernatant had been reduced to < 80 ml, the preparation was desalted on a large Sephadex G-10 column (~600 ml bed volume) eluted with distilled water. The carbohydrate-positive fractions (determined by the anthrone colorimetric assay[33]) with low conductivity (low salt) were pooled and then dried under reduced pressure at 40 °C. Methanol (200 ml) was added to the residue and evaporated, twice. Then methanol containing 10% acetic acid (300 ml) was added and dried and this procedure was repeated three times. Finally, methanol (200 ml) was added and the residue was dried three times. These evaporations were performed to volatilize the methyl ester of borate. The residue was dissolved in distilled water, the *p*H adjusted to 7.0 and the solution lyophilized.

It would be difficult to study the biochemical and molecular genetic phenomena underlying induced morphogenesis in these tissues because auxin and cytokinin are both pleiotropic growth regulators; that is, they have many effects on plant tissues besides influencing morphogenesis. Furthermore, altering the *p*H of the culture medium is also likely to have effects on such diverse factors as the biochemical properties of the cell wall and the ability of the plasma membrane to perceive and transport plant growth regulators[13,14], salts[15] and other metabolites[16,17]. We hypothesize that auxin, cytokinin and the *p*H of the culture medium affect the release of, processing of and/or responsiveness to chemical messages, each of which is not broadly pleiotropic and each of which regulates a delineated set of biochemical processes that regulate morphogenesis[4,18–20]. The experiments described here were designed to obtain evidence for the existence of chemical messages that have more specific effects than do the classical plant growth regulators.

Certain oligosaccharides, derived by means of fragmenting plant cell wall polysaccharides, are chemical messages that have been shown to be capable of regulating physiological processes in plants (for example, refs 18, 21–26 and S. Doares, A.G.D. and P.A., unpublished results). Oligosaccharides capable of regulating physiological processes have been called oligosaccharins[27]. The discovery that oligosaccharins can control gene expression in plants motivated us to design the present experiments (see Table 1, methods), which demonstrate that oligosaccharins derived from the cell walls of suspension-cultured sycamore cells can regulate the morphogenesis of the thin cell-layer explants of tobacco.

The tobacco explants, when grown in medium I at *p*H 3.8, formed an average of 10 flowers each (Table 1). Adding 1 $\mu g\ ml^{-1}$ of EPG fragments (oligosaccharins released by endo-α-1,4-polygalacturonase treatment of sycamore cell walls) inhibited flowering by 87% and induced the explants to form many vegetative buds over the entire surface area. (Flowers formed only on the basipetal end of the explants.) In medium II at *p*H 5.0, in the absence of added EPG fragments, the explants developed, not organs, but rather, large amounts of undifferentiated callus. The formation of callus was inhibited by 1 or 10 $\mu g\ ml^{-1}$ of EPG fragments and the formation of large numbers of vegetative buds was induced by EPG fragments. In medium II at *p*H 6.0, in the absence of added fragments, the explants formed vegetative buds. However, formation of buds was almost completely inhibited and, instead, formation of flowers was induced by adding 1 $\mu g\ ml^{-1}$ of EPG fragments.

The base-solubilized (B) fragments have an effect similar to that of the EPG fragments in medium I at *p*H 3.8 (Table 2). Although the B fragments did induce formation of vegetative buds and inhibit flowering, 10 $\mu g\ ml^{-1}$ of the B fragments was required, compared with the 1 $\mu g\ ml^{-1}$ of EPG fragments required in the previous example. Furthermore, B fragments caused the explants to form floral shoots, rather than flowers, on the same explants that formed vegetative buds. In medium II at *p*H 6.0, these B fragments, like the EPG fragments, inhibited vegetative bud formation; but the B fragments induced roots, rather than flowers, to form.

Table 2 Effect of B fragments on morphogenesis of tobacco explants

	Concentration of B fragments ($\mu g\ ml^{-1}$)	Flowers	Floral shoots	Vegetative buds	Roots	Callus
Medium I	0	600*	0	0	0	0
*p*H 3.8	1	600	300	27	0	0
	10	0	300	1,200	0	0
Medium II	0	0	0	0	0	100%
*p*H 5.0	1	0	0	0	300	100%
	10	0	0	0	420	100%
*p*H 6.0	0	0	0	1,800	0	0
	1	0	0	0	420	100%
	10	0	0	0	450	100%

* See Table 1 legend.

We estimate the concentration of active oligosaccharins in the EPG and B fragment mixtures to be 10^{-8} or 10^{-9} M, which matches the specific activity observed for other oligosaccharins[22,28]. Also, like the other oligosaccharins[21], the EPG and B fragments exhibit activity over a rather narrow (one or two orders of magnitude) range of concentration; the morphogenetic effects disappear when the concentration is too high or too low (unpublished data). Furthermore, the EPG and B fragments exhibit the described effects in the presence of 30 mg ml^{-1} of glucose.

Thus the effect of the EPG and B fragments on morphogenesis does not result from a nonspecific carbohydrate effect. The ability of oligosaccharins to regulate morphogenesis in tobacco explants makes apparent the possibility that these fragments are *in situ* regulators of morphogenesis. Our goal is to ascertain whether this is, or is not, the case.

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1. Skoog, F. & Miller, C. O. *Symp. exp. Biol.* **15**, 118-131 (1957).
2. Cousson, A. & Tran Thanh Van, K. *Pl. Physiol.* **72**, 33-36 (1983).
3. Tran Thanh Van, K. in *Regulation of Morphogenesis in Plant Tissue Culture and its Biotechnological Application* (eds Barz, W., Reinhard, E. & Zenk, M. H.) 367-385 (Springer, Berlin, 1977).
4. Cousson, A. & Tran Thanh Van, K. *Physiol. Pl.* **51**, 77-84 (1981).
5. Cousson, A., Toubart, P. & Tran Thanh Van, K. *Bull. Soc. bot. Fr.* (in the press).
6. Aghion-Pratt, D. *Physiol. Vég.* **3**, 229-303 (1965).
7. Chlyah, H. *Pl. Physiol.* **54**, 341-348 (1974).
8. Chlyah, H. *Pl. Physiol.* **62**, 482-485 (1978).
9. Tran Thanh Van, M. *Planta* **115**, 87-92 (1973).
10. Tran Thanh Van, K. *Int. Rev. Cytol.* Suppl. 11A, 175-194 (1980).
11. Tran Thanh Van, K. *A. Rev. Pl. Physiol.* **32**, 291-311 (1981).
12. Tran Thanh Van, K., Dien, D. T. & Chlyah, A. *Planta* **119**, 149-159 (1974).
13. Rubery, P. H. & Sheldrake, A. R. *Nature new Biol.* **224**, 285-288 (1973).
14. Rubery, P. H. & Sheldrake, A. R. *Planta* **118**, 101-121 (1974).
15. Martin, S. M. & Rose, D. *Can. J. Bot.* **54**, 1264-1270 (1976).
16. Colombo, R., De Michaelis, M. I. & Lado, P. *Planta* **138**, 249-256 (1978).
17. Komor, E. & Tanner, W. *Biochim. biophys. Acta* **241**, 170-179 (1971).
18. Darvill, A. G. & Albersheim, P. *A. Rev. Pl. Physiol.* **35**, 243-275 (1984).
19. Darvill, A. G. *et al. J. Sci.* Suppl. (in the press).
20. McNeil, M., Darvill, A. G., Fry, S. C. & Albersheim, P. *A. Rev. Biochem.* **53**, 625-663 (1984).
21. Gollin, D. J., Darvill, A. G. & Albersheim, P. *Biol. Cell* **51**(2), (in the press).
22. York, W. S., Darvill, A. G. & Albersheim, P. *Pl. Physiol.* **75**, 295-297 (1984).
23. Nothnagel, E. A., McNeil, M., Albersheim, P. & Dell, A. *Pl. Physiol.* **71**, 916-926 (1983).
24. Jin, D. J. & West, C. A. *Pl. Physiol.* **74**, 989-992 (1984).
25. Fry, S. C., Darvill, A. G. & Albersheim, P. *Br. Pl. Growth Regulator Group Monogr.* **9**, 33-43 (1983).
26. Yamazaki, N., Fry, S. C., Darvill, A. G. & Albersheim, P. *Pl. Physiol.* **72**, 864-869 (1983).
27. Albersheim, P. *et al.* in *Structure and Function of Plant Genomes* (eds Ciferri, O. & Dure, L. III) 293-312 (Plenum, New York, 1983).
28. Sharp, J. K., Valent, B. & Albersheim, P. *J. biol. Chem.* **259**(18), 11312-11320 (1984).
29. Murashige, T. & Skoog, F. *Physiologia Pl.* **15**, 473-497 (1962).
30. English, P. D., Maglothin, A., Keegstra, K. & Albersheim, P. *Pl. Physiol.* **49**, 293-297 (1972).
31. Talmadge, K. W., Keegstra, K., Bauer, W. D. & Albersheim, P. *Pl. Physiol.* **51**, 158-173 (1973).
32. Darvill, A. G., McNeil, M. & Albersheim, P. *Pl. Physiol.* **62**, 418-422 (1978).
33. Dische, Z. in *Methods in Carbohydrate Chemistry* Vol. 1 (eds Whistler, R. L. & Wolfrom, M. L.) 478-481 (Academic, New York, 1962).

Assembly in *E. coli* of a functional multi-subunit ribulose bisphosphate carboxylase from a blue-green alga

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The enzyme ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBPCase) has a pivotal role in photosynthetic organisms, catalysing the first reactions of the interlocked but opposing pathways of photosynthesis and photorespiration[1,2]. The agricultural interest in RuBPCase centres on the possibility of altering the structure of the enzyme to improve net photosynthetic yield, but such studies are hampered by the structural complexity of the hexadecameric holoenzyme in plants which prevents *in vitro* dissociation and reconstitution, apparently due to the properties of the large subunits[3,4]. We have chosen to express the large and small subunit genes of the cyanobacterium *Synechococcus* PCC6301 in *Escherichia coli* to develop an experimental system in which structural changes introduced into RuBPCase polypeptides could be assessed following subunit assembly. The cyanobacteria (blue-green algae) are autotrophic prokaryotes which have a plant-like photosynthetic mechanism and RuBPCase structure[5,6]. We report here the synthesis of an active RuBPCase in *E. coli* which contains both large and small subunit polypeptides.

The genes for the large subunit (L) and small subunit (S) of RuBPCase from *Synechococcus* have been identified on a 2.17-kilobase (kb) *Pst*I fragment of DNA[7,8]. To obtain expression of both L and S genes, this *Pst*I fragment was cloned into the *Pst*I site of the plasmid pLa2311 (ref. 9) downstream from a λ P_L promoter, to yield plasmid pSV55 (Fig. 1*a*). To demonstrate that RuBPCase polypeptides could be synthesized following derepression of P_L, a culture of *E. coli* cells containing pSV55 was incubated at 41 °C and a protein extract prepared. Electrophoresis of the total cell proteins in polyacrylamide gels containing SDS, followed by Western blotting using anti-wheat RuBPCase serum, revealed the presence of two immunoreacting polypeptides (Fig. 1*b*, lane 2). The smaller polypeptide has a relative molecular mass (M_r) of 52,000 and co-migrates with L present in maize leaf extracts (Fig. 1*b*, lane 3). The larger polypeptide of M_r 70,000–72,000 is a fusion protein between the 181 N-terminal amino acids of β-lactamase[10] and L. The detection of both polypeptides was dependent on transcription from P_L; in repressed conditions neither was observed (Fig. 1*b*, lane 1), nor if the orientation of the genes was reversed with respect to P_L (data not shown). *Synechococcus* S could not be identified in Fig. 1*b*, lane 2, when using anti-wheat RuBPCase serum because of the greater evolutionary divergence of S compared with L polypeptides. To demonstrate S synthesis, derepressed *E. coli* cells containing pSV55 were labelled with ^{35}S-methionine and the assembled soluble enzyme complex was separated from unassembled subunits by gel filtration, then reacted with anti-wheat RuBPCase. The immune complex was adsorbed to protein A–Sepharose, thus trapping assembled S via binding to L, which in turn interacts with IgG and protein A. Figure 1, lane 4, shows that both L and S are present together in a subunit complex excluded from a Biogel P-300 column (exclusion limit 400,000 M_r).

It has been shown that the presence of both S and L are essential for the catalytic function of *Synechococcus* RuBPCase[11]. The soluble fractions of sonicated *E. coli* cells containing pSV55, with or without induction, were prepared and used to measure RuBPCase activity. Figure 2*a* shows the results of assaying for RuBP-dependent incorporation of $^{14}CO_2$ into acid-stable products. Induced cell extracts show a linearity of ^{14}C incorporation with time and a specific activity of 8 nmol CO_2 fixed per min per mg soluble protein at 30 °C. In contrast, the uninduced cell extract contains no detectable RuBPCase activity. The RuBPCase activity present in induced cell extracts can be inhibited by the addition of anti-wheat RuBPCase serum before assay (Fig. 2*b*). To confirm that RuBPCase activity was dependent on the presence of S, the following deletion experiment was devised. The 2.17-kb *Pst*I fragment was transferred from pSV55 to the *Pst*I site of pUC9 (ref. 12) in the appropriate orientation for the L and S genes to be transcribed from the *lac* promoter. The resulting plasmid, pDB50, when induced with isopropyl β-D-thiogalactoside, gave a specific activity for RuBPCase of 14 nmol CO_2 fixed per min per mg soluble protein at 30 °C when assayed as described in Fig. 2 legend. Plasmid

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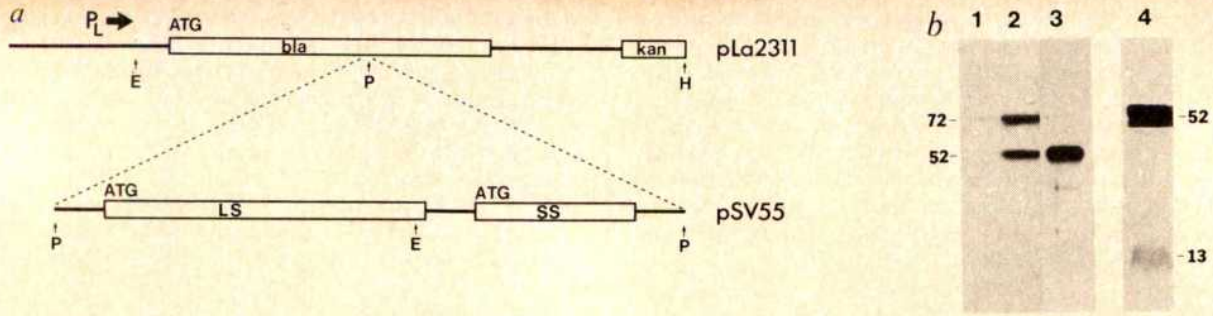


Fig. 1 *a*, Construction of RuBPCase expression plasmids. There are asymmetrical *Eco*RI sites in the vector and insert. The plasmid in which the insert is orientated for RuBPCase gene transcription from P_L is designated pSV55. The position of the *Pst*I (P), *Eco*RI (E) and *Hin*dIII (H) sites are indicated, together with the large subunit (LS), small subunit (SS) and β-lactamase (*bla*) genes with their initiator methionine (ATG) marked. The direction of transcription from the P_L promoter is shown with an arrow. *b*, Analysis of RuBPCase polypeptide synthesis in M5219 cells containing pSV55 plasmid. Lane 1, total protein of uninduced pSV55 (Western blot); lane 2, total protein of induced pSV55 (Western blot); lane 3, total maize leaf protein (Western blot); lane 4, immunoadsorbed pSV55-encoded L and S polypeptides labelled with ^{35}S *in vivo*.

Methods. *a*, The *Synechococcus* RuBPCase L and S genes are present on a 2.17-kb *Pst*I fragment in the clone pLS506 (ref. 8). The pLS506 plasmid was digested with *Pst*I and ligated into the P_L expression plasmid pLa2311 (ref. 9), previously linearized with *Pst*I at a site within the ampicillin-resistance gene. The kanamycin-selectable marker on pLa2311 was retained intact. The ligation mixture was transformed into strain M5219 [M72*lacZ*am*trp*amSm^R(λ*bio*252*c*I857ΔH1)] (ref. 9) and kanamycin-resistant colonies selected. Ampicillin-sensitive colonies containing the 2.17-kb *Pst*I insert were digested with *Eco*RI to identify clones with the two possible orientations of the fragment. *b*, To detect LS synthesis, M5219 cells containing pSV55 were grown at 30 °C in 100 ml of L broth containing 50 μg ml^{-1} kanamycin to $A_{600} = 0.4$. Cells were induced at 45 °C for 10 min and then cultured at 41 °C for 2 h with vigorous shaking. Uninduced controls were maintained at 30 °C. Cells were collected by centrifugation and incubated for 30 min on ice in 0.5 ml of 10 mM Tris-HCl *p*H 8.0, 10 mM EDTA, 1 mg ml^{-1} lysozyme. The solution was sonicated for 30 s, mixed with 0.5 ml 10% (w/v) SDS and incubated at 25 °C for 10 min. A soluble fraction was recovered after centrifugation in an Eppendorf microcentrifuge for 15 min. Maize leaf proteins were isolated by grinding 1 g of young tissue with 3 ml of 100 mM Tris-HCl *p*H 8.0, 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM EDTA, 1 mM β-mercaptoethanol and 2% (w/v) insoluble polyvinylpyrrolidone (PVP; buffer A). The homogenate was clarified using an Eppendorf microcentrifuge for 5 min. Soluble proteins from bacterial and leaf cells were heated in SDS loading buffer and electrophoresed on a 15% polyacrylamide gel containing SDS[19]. Polypeptides were transferred from the gel to nitrocellulose[20], the filters preincubated with 1.5% (w/v) gelatin in 10 mM Tris-HCl *p*H 7.5, 0.35 M NaCl, for 2 h, and then incubated with 25 μl anti-wheat RuBPCase serum overnight in 25 ml 10 mM Tris-HCl *p*H 7.5, 0.15 M NaCl, 0.1% deoxycholate, 0.1% SDS and 1% Triton X-100 (RIA buffer). After washing with RIA buffer, the filter was incubated with 0.5 μCi ^{125}I-protein A for 2 h and then washed in RIA buffer before autoradiography. To detect SS synthesis, a 25-ml culture of K-12ΔH1Δ*trp* cells[9] containing pSV55 was grown in M9 medium containing glucose, tryptophan, biotin and thiamine-HCl to $A_{600} = 0.5$ at 30 °C. The culture was shifted to 42 °C and after 5 min 2 mCi ^{35}S-methionine were added. After 1.5 h at 42 °C, the cells were collected by centrifugation, resuspended in 0.5 ml 25% (w/v) sucrose, 50 mM Tris-HCl *p*H 8.0, 10 mM EDTA, 1 mg ml^{-1} lysozyme for 15 min at 0 °C, and then lysed by the addition of an equal volume of 50 mM Tris-HCl *p*H 8.0, 100 mM NaCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 1 mM β-mercaptoethanol and 0.1% (v/v) Triton X-100 (buffer B). Phenylmethylsulphonyl fluoride was added to 1 mM, the lysate sheared through a hypodermic syringe and then centrifuged at 100,000*g* for 1 h. The supernatant (1 ml) was passed through a 0.9 × 27 cm column of Biogel P-300 (50–100 mesh) in buffer B at 4 °C. The excluded column fractions ($M_r \geqq 400{,}000$) were incubated with anti-wheat RuBPCase and then adsorbed to protein A–Sepharose and washed as before[21,22] in non-denaturing conditions. The adsorbed polypeptides were boiled in SDS sample buffer, resolved by electrophoresis on a 20% polyacrylamide gel containing SDS[19] and fluorographed[23]. Gels were calibrated with ^{14}C-labelled markers as before[21,22].

pDB50 was digested with *Pst*I and partially with *Hin*dIII (see ref. 8) and a resulting 1.7-kb fragment containing only the L gene transferred to the *Pst*I/*Hin*dIII sites of pUC8 (ref. 12), such that the L gene was transcribed from the *lac* promoter. Induction of the resulting plasmid pDB53 did not yield any detectable RuBPCase activity, indicating that S is required for the active enzyme complex to form in *E. coli*. Western blotting confirmed that L synthesis was still directed by pDB53 (data not shown).

For further probing of the active site, the transition-state analogue 2-carboxyarabinitol 1,5-bisphosphate (CABP) was used. This binds to the activated ternary complex $E.CO_2.Me^{2+}$ to give a stable quaternary complex of $E.CO_2.Me^{2+}.CABP$ (ref. 1). A mixture of ^{14}C-labelled diastereomers CABP and 2-carboxyribitol 1,5-bisphosphate (CRBP) were synthesized[13] and then incubated with soluble extracts of control *E. coli* cells, induced cells containing pSV55, or purified spinach RuBPCase. The extracts were passed through affinity columns containing immobilized anti-wheat RuBPCase and the immunoadsorbed protein was eluted with 8 M urea[14]. Column fractions were counted for ^{14}C isotope content and aliquots analysed by Western blotting. In Fig. 3*a*, labelled peaks can be seen for the spinach RuBPCase and the pSV55 extract, but not for the control strain. The labelled analogue binding, together with the antibody column, has therefore allowed the detection of specific binding to the active site of the *Synechococcus* enzyme synthesized in *E. coli*. Figure 3*b* confirms that the protein adsorbed to the column contains the L polypeptide.

RuBPCase isolated from *Synechococcus* has a M_r of 530,000 (ref. 6) with subunit M_rs of 52,470 for L[5] and 14,000 for S[15], and a hexadecameric structure of L_8S_8. To analyse the structural complexity of the enzyme synthesized in *E. coli*, soluble protein extracts from induced cells containing pSV55 were centrifuged on sucrose gradients. The position of the enzyme in the gradient was determined either by RuBPCase activity or by prior labelling of the active site with ^{14}C-labelled CABP/CRBP. The protein samples layered on the gradients contained β-galactosidase as an internal marker, and gradients centrifuged in parallel contained spinach RuBPCase (M_r 550,000), β-galactosidase (465,000), aldolase (160,000) and ovalbumin (43,000). Figure 4*a*, *b* shows that the size of RuBPCase synthesized in *E. coli* is similar to spinach RuBPCase. Sucrose gradient fractions analysed by SDS-gel electrophoresis and Western blotting using anti-wheat RuBPCase serum revealed the presence of the *Synechococcus* L polypeptide in the fractions containing the RuBPCase peak of activity (Fig. 4*c*). There was also some β-lactamase/L fusion protein in the lower part of the gradient, although most of this 72,000-M_r protein is located in the membrane fraction, presumably because of initiation of export by the β-lactamase signal sequence[16]. It does not necessarily follow that a hexadecameric structure is formed in *E. coli*, because the RuBPCase synthesized in the bacteria and spinach leaves is of a similar size, as determined by sucrose gradient centrifugation and by electrophoresis on non-denaturing gels (data not shown). The large size indicates that the L_8 structure is probably formed, but an L_8S_n complex where n is less than 8 would be difficult to resolve because of the small size of S. Although S must be present to obtain an active enzyme[15], we believe that the S

binding sites on the L octamer may not be fully saturated because of two observations. First, we can estimate from ^{14}C-labelled CABP/CRBP binding to L_8S_n complexes on sucrose gradients that these structures comprise about 2% of the *E. coli* soluble proteins and yet the specific activity of RuBPCase in crude extracts is lower than expected. Second, liquid scintillation counting of ^{35}S present in the L and S polypeptides isolated from SDS-polyacrylamide gels (Fig. 1*b*, lane 4) indicates that L_8S_n structures are formed where $n = 2$ or 3. This problem is likely to be resolved by increased production of S relative to L using suitable plasmids.

Previous work has shown that active RuBPCase can be synthesized in *E. coli* using genes from purple non-sulphur bacteria to give either a dimeric L_2 (ref. 17) or a hexameric L_6 (ref. 18) enzyme. These enzymes, however, are atypical in that an S polypeptide is not required for activity. The most common form of the enzyme is the L_8S_8 structure found in plants, and in prokaryotes possessing a Calvin cycle. In the L_8S_8 structures, the presence of S is essential for activity, based on evidence from *Synechococcus*[15] and from our data presented here in which deletion of the S gene abolishes RuBPCase activity. A suggested role for S is that its binding to L induces a conformational change that promotes activity, and so it has an essential but indirect role in catalysis[15]. This report, describing the assembly in *E. coli* of an L_8 structure containing S subunits to enable the enzyme to fix CO_2, represents one of the most complex macromolecular structures to be synthesized using recombinant DNA techniques. The RuBPCase activity is presumably

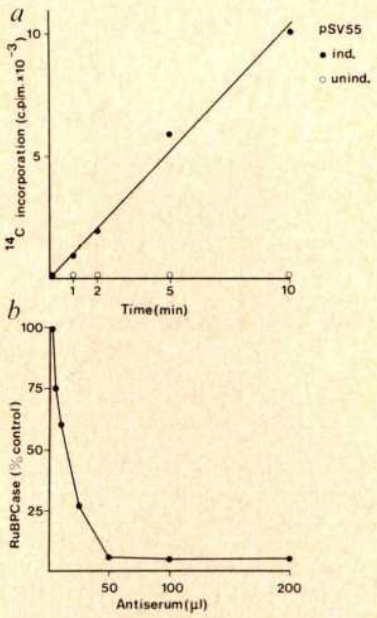


Fig. 2 RuBPCase activity in induced cell extracts of M5219 containing pSV55, and antibody inhibition of enzyme activity. *a*, The linear incorporation of ^{14}C into acid-stable material using RuBP as substrate and plasmid pSV55 in either induced (●) or uninduced (○) M5219 cells. *b*, Increasing inhibition of RuBPCase activity in induced pSV55-containing cell extracts as increasing amounts of anti-wheat RuBPCase serum are added.

Methods. To measure RuBPCase activity, cells were grown in 800 ml of L broth with 50 μg ml^{-1} kanamycin, induced and collected as described in Fig. 1*b*. Cells were resuspended in 1 ml of 100 mM Tris-HCl *p*H 8.0, 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM EDTA and 1 mM β-mercaptoethanol. Cells were broken by sonication and the extract clarified at 100,000*g* for 1 h. RuBP-dependent ^{14}C incorporation into acid-stable material was assayed using 100-μl aliquots containing 2.7 mg ml^{-1} protein at 30 °C (ref. 11). The activity in an uninduced control was also measured. For antibody inhibition studies, 100-μl aliquots of induced soluble pSV55 extracts were incubated with various amounts of anti-wheat RuBPCase serum overnight at 4 °C. The samples were centrifuged for 15 min in an Eppendorf microcentrifuge and the supernatant used for RuBPCase assay[11].

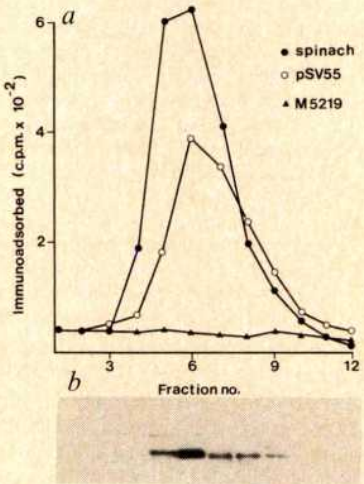


Fig. 3 Affinity column binding of RuBPCase labelled with the transition state substrate analogue ^{14}C-CABP. *a*, The ^{14}C-CABP elution profile of spinach RuBPCase (●), induced pSV55 encoded RuBPCase (○) and control M5219 cell extracts (▲). *b*, Identification of L polypeptide in the column fractions from the pSV55 extract using Western blotting.

Methods. Cell extracts were prepared by sonication as in Fig. 2, using either induced M5219 cells containing pSV55 or control M5219 cells. In addition, purified spinach RuBPCase was used as a control. All three samples in 50 ml of 50 mM Tris-HCl *p*H 8.0, 20 mM $MgCl_2$, 1 mM EDTA, 1 mM β-mercaptoethanol and 25 mM $NaHCO_3$ were incubated for 15 min at 30 °C. ^{14}C-CABP was added to a final concentration of 10 μM (ref. 3) and incubation continued for 1 h. NaCl was added to 0.1 M and the extracts passed through 1 × 5 cm columns of immobilized anti-wheat RuBPCase[14] equilibrated with the incubation buffer. The columns were washed with 50 mM Tris-HCl *p*H 8.0, 1 M NaCl, 20 mM $MgCl_2$, 1 mM EDTA, 1 mM β-mercaptoethanol, 0.1% Triton X-100 (25 ml), then with incubation buffer (50 ml). Immunoadsorbed protein was eluted with 8 M urea[14] and 0.5-ml fractions collected. Half of each fraction was counted using liquid scintillation counting, the remainder was precipitated with trichloroacetic acid, electrophoresed on a SDS/15% polyacrylamide gel, and probed by Western blotting.

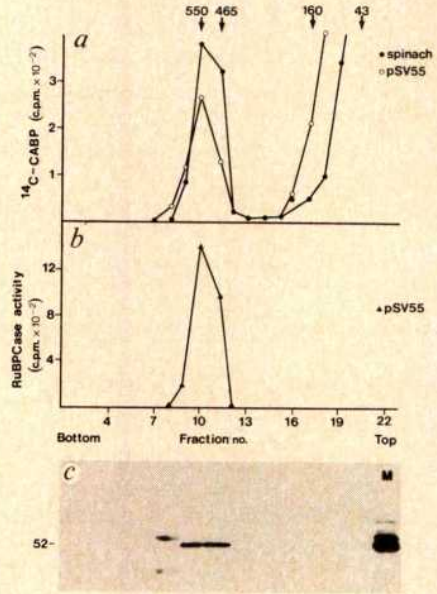


Fig. 4 Velocity sedimentation of RuBPCase in sucrose gradients. *a*, Gradient profile of ^{14}C-CABP-treated protein using spinach RuBPCase (●) or induced pSV55-containing cells (○). *b*, Distribution of RuBPCase activity on a sucrose gradient using induced pSV55-containing cells (▲). *c*, Western blot of the gradient fractions shown in panel *b*. The M track is a maize leaf extract used as a marker. M_r markers shown by arrows.

Methods. Soluble protein extracts were prepared as described in Fig. 2 from induced and uninduced M5219 cells containing pSV55. β-Galactosidase was added as a marker to these extracts and 500 μl was layered on to an 11-ml linear 7–25% sucrose gradient in 0.1 M Tris-HCl *p*H 8.0, 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM EDTA, 1 mM mercaptoethanol. The gradients were centrifuged for 24 h at 25,000 r.p.m. and 4 °C in a Sorvall TST41 swinging bucket rotor. Fractions (0.5 ml) were collected from the bottom of the tube and assayed for RuBPCase activity[11]. Alternatively, the protein extract was incubated with ^{14}C-CABP at 10 μM concentration for 1 h before centrifuging. In this case, the gradient fractions were counted directly for ^{14}C content. Aliquots of fractions were also analysed by electrophoresis and Western blotting to locate the position of the L polypeptide on the gradient. All gradients had an internal marker, β-galactosidase (M_r 465,000), that could be assayed directly[24]. In addition, identical calibration gradients were centrifuged at the same time containing spinach RuBPCase (M_r 550,000), β-galactosidase (465,000), aldolase (160,000) and ovalbumin (43,000).

expressed by LS pairs present in the L_8S_n structures irrespective of the value of *n*. This system should provide an opportunity to gain further understanding of the assembly mechanisms and the importance of subunit contact sites in forming and maintaining the structure of the holoenzyme.

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Note added in proof: By increasing the expression of S, we find that RuBPCase activity is also enhanced, suggesting that the S binding sites on the L octamer can be increasingly saturated by S overproduction.

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1. Miziorko, H. M. & Lorimer, G. H. *A. Rev. Biochem.* **52**, 507-535 (1983).
2. Ellis, R. J. & Gatenby, A. A. *The Genetic Manipulation of Plants and its Application to Agriculture* (eds Lea, P. J. & Stewart, G. R.) 41-60 (Oxford University Press, 1984).
3. Gatenby, A. A. *Eur. J. Biochem.* **144**, 361-366 (1984).
4. Voordouw, G., van der Vies, S. M. & Bouwmeister, P. P. *Eur. J. Biochem.* **141**, 313-318 (1984).
5. Shinozaki, K., Yamada, C., Takahata, N. & Sugiura, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4050-4054 (1983).
6. Andrews, T. J., Abel, K. M., Menzel, D. & Badger, M. R. *Archs Microbiol.* **130**, 344-348 (1982).
7. Shinozaki, K. & Sugiura, M. *Nucleic Acids Res.* **11**, 6957-6964 (1983).
8. Reichelt, B. Y. & Delaney, S. F. *DNA* **2**, 121-129 (1983).
9. Remaut, E., Stanssens, P. & Fiers, W. *Gene* **15**, 81-93 (1981).
10. Sutcliffe, J. G. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3737-3741 (1978).
11. Andrews, T. J. & Abel, K. M. *J. biol. Chem.* **256**, 8445-8451 (1981).
12. Vieira, J. & Messing, J. *Gene* **19**, 259-268 (1982).
13. Rabin, B. R., Shaw, D. F., Pon, N. G., Anderson, J. M. & Calvin, M. *J. Am. chem. Soc.* **80**, 2528-2532 (1958).
14. Gatenby, A. A. & Cocking, E. C. *Pl. Sci. Lett.* **10**, 97-101 (1977).
15. Andrews, T. J. & Ballment, B. *J. biol. Chem.* **258**, 7514-7518 (1983).
16. Kadonaga, J. T. *et al. J. biol. Chem.* **259**, 2149-2154 (1984).
17. Somerville, C. R. & Somerville, S. C. *Molec. gen. Genet.* **193**, 214-219 (1984).
18. Quivey, R. G. & Tabita, F. R. *Gene* **31**, 91-101 (1984).
19. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
20. Vaessen, R. T. M. J., Kreike, J. & Groot, G. S. P. *FEBS Lett.* **124**, 193-196 (1981).
21. Gatenby, A. A., Castleton, J. A. & Saul, M. W. *Nature* **291**, 117-121 (1981).
22. Gatenby, A. A. & Castleton, J. A. *Molec. gen. Genet.* **185**, 424-429 (1982).
23. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83-88 (1974).
24. Miller, J. H. *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, New York, 1972).

Renal cytochrome P450-related arachidonate metabolite inhibits $(Na^+ + K^+)$ATPase

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The function of the nephron, the anatomical unit of the kidney, is segmented; at least 12 segments have been identified that differ in their morphology, transport properties and hormonal responsiveness[1]. The medullary portion of the thick ascending limb of the loop of Henle (mTALH) has one of the highest concentrations of $(Na^+ + K^+)$ATPase found in mammalian tissues[2], reflecting the importance of this nephron segment in the regulation of extracellular fluid volume, as active sodium transport is driven by $(Na^+ + K^+)$ATPase[1]. Here, in cells derived primarily from the mTALH of the outer medulla of rabbit kidney, we have identified a cytochrome P450-dependent monooxygenase system[3] which metabolizes arachidonic acid to two biologically active oxygenated products; one of the products inhibits $(Na^+ + K^+)$ATPase and the other relaxes blood vessels. We report that formation of these oxygenated arachidonate metabolites is stimulated by arginine vasopressin (AVP) and salmon calcitonin (SCT).

Male New Zealand White rabbits (3.0-3.5 kg) were anaesthetized with sodium pentobarbital (30 mg per kg), and the inner stripe of the outer medulla excised, cut into small pieces and trypsinized for 20 min in trypsin-EDTA (1×) plus 0.1% bovine serum albumin. A single-cell suspension of outer medullary cells was prepared and separated into several fractions by centrifugal elutriation[3,4]. Cells derived mainly from the mTALH were eluted at a flow rate of 19 ml min^{-1} at 2,000 r.p.m., at 20 °C[3]. Over 90% of the cells were viable as determined by Trypan blue exclusion, electron microscopic appearance and metabolic activity. After cell separation, ~80% of the cells proved to be mTALH in origin, based on electron microscopic criteria and immunofluorescent localization of Tamm-Horsfall protein, a specific marker for mTALH cells[5]. The remainder were cells derived from proximal tubules and collecting ducts. The specific activity of alkaline phosphatase, a marker for proximal tubule cells[6], decreased threefold after separation of mTALH cells from outer medullary cells, in association with a fourfold increase in the ability of the separated mTALH cells to metabolize arachidonic acid to oxygenated metabolites.

Incubation of mTALH cells with ^{14}C-labelled arachidonic acid resulted in formation of oxygenated metabolites, identified as peaks I and II (see Fig. 1), which accounted for 30-40% of the recovered radioactivity. Formation of the arachidonate

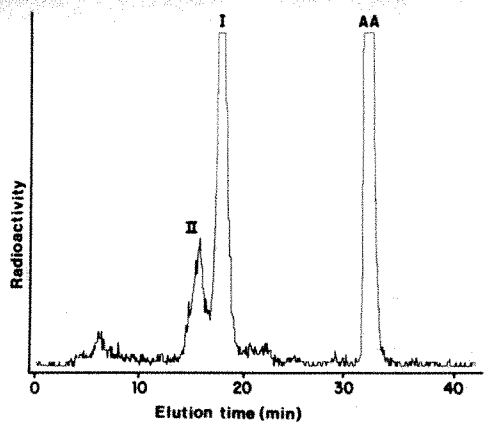


Fig. 1 Reverse-phase chromatography of arachidonic acid metabolites formed by mTALH cells. The retention times of peaks I and II were 18.2 and 15.7 min, respectively, while those for the monohydroxyeicosatetraenoic acids (mono-HETEs) in this HPLC separation were as follows: 15-HETE, 19 min; 11-HETE, 20 min; 12-HETE, 21 min; 9-HETE, 22 min; 5(*S*),12(*S*)-diHETE, 17.5 min.

Methods. Cells (3×10^6 ml^{-1}) were incubated in phosphate-buffered saline (PBS) with 0.4 μCi ^{14}C-arachidonic acid (7 μM; specific activity 56 mCi $mmol^{-1}$) at 37 °C for 30 min. The reaction was stopped by acidification to *p*H 4.0, the cells were removed by centrifugation and the media extracted with ethyl acetate. Arachidonic acid metabolites were separated by HPLC on a μ Bondapack C_{18} column, using a linear solvent gradient ranging from water:acetonitrile (1:1, containing 0.1% acetic acid) to 100% acetonitrile (containing 0.1% acetic acid); the rate of change was 1.25% per min at a flow rate of 1 ml min^{-1}. Radioactive products were detected using a radioactive flow detector. Fractions containing peaks I and II were collected separately, methylated with a freshly prepared solution of diazomethane in ether and subjected to further purification on normal-phase HPLC using a μ Porasil column and solvent system comprising hexane/isopropanol/acetic acid (98:2:0.1) at a flow rate of 1 ml min^{-1}. The purified methylated products were further derivatized to the trimethylsilyl derivatives and subjected to GC-MS analysis, using a Hewlett-Packard HP-5985A GC-MS system. The samples were separated on a glass column (1% SE-30). Operational conditions of the GC-MS were: injection port 250 °C; initial temperature of 180 °C increasing at a rate of 8 °C min^{-1} up to 250 °C; interface 275 °C; ion source 300 °C and electron impact energy of 70 eV. GC-MS analysis revealed that each peak separated by HPLC contained more than one compound. A major compound associated with peak II can be defined as the methyl ester-trimethylsilyl derivative of 11,12-dihydroxyeicosatrienoic acid because the fragments at $m/z = 496$ (M^+), 465 ($M^+ - 31$), 406 ($M^+ - 90$), 385 ($M^+ - 111$), 315 ($M^+ - 181$), 295 and 213 are characteristic of this structure. A major compound associated with peak I has prominent fragments at $m/z = 316$ ($M^+ - 18$), 303 ($M^+ - 31$), 233, 190 and 176, suggesting the presence of the methyl ester derivative of 5,6-epoxyeicosatrienoic acid.

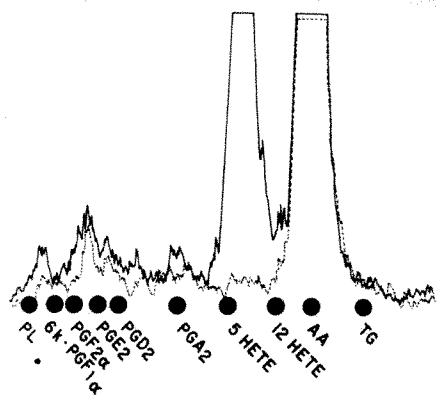


Fig. 2 Arachidonic acid metabolism by a broken cell preparation of mTALH cells. Cells were lysed by two cycles of freeze-thaw with liquid nitrogen in distilled water, then adjusted to PBS, *p*H 7.4. Broken cell preparations (200 μl, 80–100 μg protein) were incubated with ^{14}C-arachidonic acid (0.2 μCi) plus 'cold' arachidonic acid (10 μM) in the presence (—) and absence (- - -) of NADPH (1 mM) at 37 °C for 30 min. The reaction was stopped by acidification to *p*H 4.0 and extracted with ethyl acetate. The final extract was subjected to TLC for separation of arachidonic acid oxygenated metabolites using the upper phase of ethyl acetate/isooctane/acetic acid/water (110:50:20:100). PL, phospholipids; 6 k-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PGE_2, PGD_2 and PGA_2, prostaglandin E_2, D_2 and A_2, respectively; TG, triglycerides; HETE, hydroxyeicosatetraenoic acid; AA, arachidonic acid. The radioactive peak appearing between the 5- and 12-HETE standards consists of peak I and peak II defined in Fig. 1. Formation of prostaglandins accounted for only 3–5% of the radioactivity recovered.

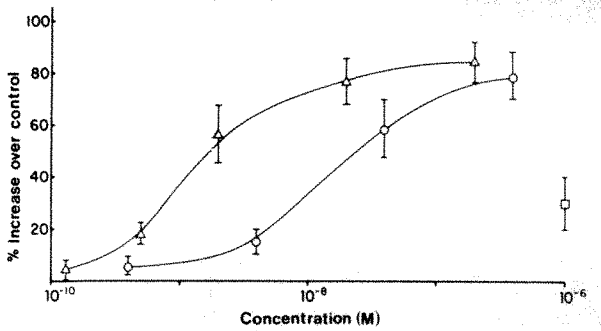


Fig. 3 Hormonal stimulation of arachidonic acid metabolism in mTALH cells prelabelled with ^{14}C-arachidonic acid (0.4 μCi per 3×10^6 cells) for 90 min at 37 °C. Within 90 min, 80–90% of ^{14}C-arachidonic acid was incorporated into phospholipids, chiefly phosphatidylcholine and phosphatidylethanolamine, and triglycerides. The cell suspension was centrifuged, then the cells were resuspended in fresh PBS and incubated for an additional 10 min with the following peptide hormones: AVP (○, 4×10^{-10}–4×10^{-7} M), SCT (△, 10^{-10}–2×10^{-7} M) and bradykinin (□, 10^{-6} M). The reaction was stopped by acidification, cells were removed by centrifugation and the media extracted with ethyl acetate. Arachidonic acid metabolites were separated by TLC as described in Fig. 2 legend; radioactive zones corresponding to peaks I and II were cut out and counted using a liquid scintillation counter. The per cent increase in the formation of peaks I and II compared with the unstimulated control value was plotted. The control value of 0.060 ± 0.007 μg per mg protein per 10 min is considerably lower than the values obtained when the 90-min period of prelabelling was omitted, as it represents release of bound radiolabelled arachidonic acid which was esterified to lipids during prelabelling. Values are mean ± s.e.m. of four experiments.

metabolites constituting peaks I and II was not affected by indomethacin[7] (3 μM), while eicosatetraynoic acid (30 μM), the acetylenic analogue of arachidonate[8], inhibited product formation by 80–90%. Separation by reverse-phase HPLC revealed that the arachidonate metabolites of peaks I and II had retention times different from those of arachidonate products of lipoxygenases, the mono- and dihydroxyeicosatetraenoic acids. Further, the lack of absorbance at 234 nm and 276 nm indicated the absence of a conjugated diene or triene structure, respectively, and suggested that the oxygenated metabolites of arachidonic acid were formed by a cytochrome P450-dependent system. At 50 μM, SKF-525A (β-diethylaminoethyldiphenylpropyl acetate hydrochloride), an inhibitor of cytochrome P450-dependent enzymes[9], reduced product formation by mTALH cells by more than 70% (from 2.52 ± 0.66 μg to 0.27 ± 0.06 μg of arachidonic acid converted per mg protein per 30 min), while induction of cytochrome P450 with 3-methylcholanthrene and β-naphthoflavone[10] (40 mg per kg per day on each of 3 days, injected intraperitoneally) increased arachidonic acid conversion to 4.17 ± 1.02 μg per mg protein per 30 min. In addition, product formation by cell-free homogenates of mTALH was dependent on the presence of NADPH (Fig. 2), suggesting that arachidonic acid metabolism in mTALH cells occurs via an NADPH-dependent cytochrome P450-linked monooxygenase pathway[11]. In view of studies based on immunofluorescent[12] and microanalytical[13] methods that have assigned the cytochrome P450-monooxygenase system to the proximal tubules, the present study, which has localized this pathway of arachidonate metabolism also in the mTALH, should be interpreted with caution.

Segmentation of nephron responsiveness to hormones may be expressed through adenylate cyclase-sensitive receptors[14]. AVP-dependent adenylate cyclase activity is restricted to the collecting ducts and mTALH[15]. Calcitonin also stimulates adenylate cyclase in the mTALH whereas adrenocorticotropic hormone, parathyroid hormone and isoprenaline do not[14]. AVP and SCT, when added to mTALH cells in concentrations ranging from 10^{-10}–10^{-7} M, stimulated release of the oxygenated arachidonate metabolites of peaks I and II by as much as twofold, depending on the dose of the peptides (Fig. 3). In contrast, concentrations of bradykinin and angiotensin II that were three orders of magnitude greater, had lesser effects on the release of oxygenated metabolites of arachidonic acid than either AVP or SCT. The phosphodiesterase inhibitor, 1-isobutyl-3-methylxanthine (10 μM), increased formation of peaks I and II almost twofold, as did dibutyryl cyclic AMP (1 mM). Parathyroid hormone and isoprenaline, which do not affect adenylate cyclase in the mTALH segment[12], had no effect on arachidonic acid metabolite formation by mTALH cells at concentrations of 10^{-10}–10^{-7} M.

Peak II proved to be a potent inhibitor of $(Na^+ + K^+)$ATPase whereas peak I was a weak inhibitor (Table 1). Peak I but not peak II relaxed rabbit pulmonary artery rings that had been precontracted with phenylephrine (5×10^{-7} M); peak I (4×10^{-8}–2.3×10^{-7} M, $n=4$) resulted in a 40–60% relaxation whereas higher doses of peak II (2.9×10^{-7}–1.2×10^{-6} M, $n=3$) had no detectable effects. Acetylcholine, used as a standard, caused a 36% relaxation of vascular rings at a dose of 5×10^{-8} M. Gas chromatography–mass spectrometry (GC–MS) analysis of peaks I and II revealed major components consistent with the structures of the 5,6-epoxide and the 11,12-diol derivatives of arachidonic acid, respectively (see Fig. 1 legend).

The results of our study indicate that renomedullary cells respond selectively to hormonal stimuli by generating arachidonic acid metabolites via a cytochrome P450-dependent mechanism, and that one of these products inhibits $(Na^+ + K^+)$ATPase. A hormone-inducible modulator of $(Na^+ + K^+)$ATPase activity has long been sought[16] and has important consequences for the regulation of extracellular fluid volume and composition. These findings also have a bearing on selective activation of the nephron by hormones via stimulation of adenylate cyclase-sensitive segments such as those of the cortical collecting ducts and mTALH by AVP[14]. Moreover, the concentrations of AVP[15] and SCT[14] that stimulate adenylate cyclase activity in mTALH correspond to concentrations of these peptides which we have shown to increase formation of arachidonic

acid metabolites in this segment, that is, 10^{-10}-10^{-8} M. As the effects of SCT and AVP on arachidonic acid metabolism are mimicked by dibutyryl cyclic AMP, the natriuretic effects of SCT[17] and AVP[18] may be mediated by adenylate cyclase-induced arachidonic acid metabolism. A natriuretic effect of AVP which is localized to mTALH[18] has been dissociated from its effect on reabsorption of water[19]. Finally, a factor which diminishes the activity of $(Na^+ + K^+)$ATPase in the mTALH has been postulated by Postnov *et al.*[20] to account for the exaggerated natriuresis in response to volume expansion in clinical and experimental hypertension. In this regard, we have demonstrated more than a twofold increase in formation of cytochrome P450-related products of arachidonic acid metabolism by TALH cells in rabbits made hypertensive by constriction of the abdominal aorta[21]. In conclusion, the cytochrome P450-dependent pathway of arachidonate metabolism in mTALH could act as a common mechanism mediating natriuresis in response to hormones as well as to a natriuretic substance(s)[22] including circulating inhibitors of $(Na^+ + K^+)$ATPase activity, levels of which are thought to be elevated in hypertension[23].

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Table 1 Effect on $(Na^+ + K^+)$ATPase of arachidonic acid metabolites formed by renomedullary cells, primarily from the thick ascending limb of rabbit kidney

	Concentration* (μM)	% Inhibition of $(Na^+ + K^+)$ATPase†
Peak I	0.05	No effect
	0.28	10
	2.60	35
Peak II	0.06	34
	0.20	65
	0.66	70

ATPase inhibition was assayed using a microsomal preparation from canine heart, prepared and treated with 6 M sodium iodide, followed by 1.5 M deoxycholate-citrate. The final pellet was dialysed overnight at 4 °C against imidazole-EDTA (25 mM-1 mM) and stored at −50 °C according to the method of Pitts and Schwartz[24]. This enzyme was 98% ouabain (1 mM)-inhibitable, with an activity of 231 μmol inorganic phosphate per mg protein per h. The 50% inhibitory concentration of ouabain for this enzyme preparation was 0.5 μM. The effects of peaks I and II on $(Na^+ + K^+)$ATPase activity, in the presence and absence of 1 mM ouabain, were measured according to the method of Martin and Doty[25]. Inorganic phosphate was measured spectrometrically by the direct addition of stannous chloride solution to an aliquot of the extract which had been diluted with ethyl alcohol-sulphuric acid solution. For the $(Na^+ + K^+)$ATPase assay, 15 μg of enzyme was incubated for 30 min at 37 °C in 1 ml of a reaction mixture containing 50 mM Tris buffer, 5 mM MgCl, 100 mM NaCl, 10 mM KCl and 0.5 mM EDTA. The reaction was started by adding ATP (5 mM) and stopped by adding 10% trichloroacetic acid. Peaks I and II (Fig. 1) are the pooled fractions (separated by reverse-phase HPLC) of oxygenated arachidonic acid metabolites formed by mTALH cells obtained from three rabbits. An identical volume of the HPLC effluent similarly extracted did not inhibit $(Na^+ + K^+)$ATPase.

* The concentrations of peaks I and II were calculated from per cent conversion of ^{14}C-arachidonic acid (after correction for extraction efficiency) to these metabolites assuming a molar ratio of 1 : 1 for each; that is, mole of arachidonic acid converts to either mole of peak I or peak II. The concentrations were estimated on the basis of a relative molecular mass of 350 for oxygenated metabolites of arachidonic acid. The estimated amount of arachidonic acid products (peaks I and II) produced by 3×10^6 cells ml^{-1} was ~400 ng in 30 min of incubation. Thus, the approximate concentration of the arachidonate metabolite(s) in the incubate was 0.1 μM, a biologically effective concentration as judged by the inhibition of $(Na^+ + K^+)$ATPase.

† Inhibition of ouabain-sensitive $(Na^+ + K^+)$ATPase. Each value is the mean of duplicate determinations of $(Na^+ + K^+)$ATPase inhibitory activity of arachidonic acid products contained in peaks I and II. Student's paired *t*-test indicated a significant difference between the activity in the two peaks ($t = 6.03$, d.f. = 2, $P < 0.05$).

1. Knepper, M. & Burg, M. *Am. J. Physiol.* **244,** F579-F589 (1983).
2. Jørgensen, P. L. *Physiol. Rev.* **60,** 864-917 (1980).
3. Ferreri, N. R., Schwartzman, M., Ibraham, N. G., Chander, P. N. & McGiff, J. C. *J. Pharmac. exp. Ther.* **231,** 441-448 (1984).
4. Grabske, R. J., Lake, S., Gledhill, B. L. & Meistrich, M. L. *J. cell. Physiol.* **86,** 177-190 (1975).
5. Hoyer, J. R., Sisson, S. P. & Vernier, R. L. *Lab. Invest.* **41,** 168-173 (1979).
6. Copenhaver, W. M., Kelly, D. E. & Wood, R. L. in *Bailey's Textbook of Histology* (eds Copenhaver, W. M., Kelly, D. E. & Wood, R. L.) 585-587 (Williams & Wilkins, Baltimore, Maryland, 1978).
7. Vane, J. R. *Nature new Biol.* **231,** 232-235 (1971).
8. Downing, D. T., Ahern, D. G. & Bachta, M. *Biochem. biophys. Res. Commun.* **40,** 218-223 (1970).
9. Testa, B. & Jenner, P. *Drug Metab. Rev.* **12,** 1-117 (1981).
10. Zenser, T. V., Mattammal, M. B. & Davis, B. B. *J. Pharmac. exp. Ther.* **207,** 719-725 (1978).
11. Capdevila, J., Marnett, L. J., Chacos, N., Prough, R. A. & Estabrook, R. W. *Proc. natn. Acad. Sci. U.S.A.* **79,** 767-770 (1982).
12. Endou, H. *Jap. J. Pharmac.* **33,** 423-433 (1983).
13. Dees, J. H., Masters, B. S. S., Muller-Eberhard, U. & Johnson, E. F. *Cancer Res.* **42,** 1423-1432 (1982).
14. Morel, F. *Am. J. Physiol.* **240,** F159-F164 (1981).
15. Imbert-Teboul, M., Chabardes, D., Montégut, M., Clique, A. & Morel, F. *Endocrinology* **102,** 1254-1261 (1978).
16. Katz, A. I. *Am. J. Physiol.* **242,** F207-F219 (1982).
17. Williams, C. C., Matthews, E. W., Moseley, J. M. & MacIntyre, I. *Clin. Sci.* **42,** 129-137 (1972).
18. Fejes-Tóth, G. & Szénási, G. *J. Physiol., Lond.* **318,** 1-7 (1981).
19. Morel, F. *Bull. biol. Fr. Belg. Suppl.* **39,** 136 (1955).
20. Postnov, Y. U., Reznikova, M. & Boriskina, G. *Pflügers Arch. ges. Physiol.* **362,** 95-99 (1976).
21. Carrara, M. C., Carroll, M. A., McGiff, J. C. & Schwartzman, M. *Br. J. Pharmac. Abstr.* (in the press).
22. deWardener, H. E. *et al. Lancet* **i,** 411-412 (1981).
23. Overbeck, H. W., Pamnani, M. B., Akera, T., Brody, T. M. & Haddy, F. J. *Circulation Res.* **38,** II-48-II-52 (1976).
24. Pitts, B. J. R. & Schwartz, A. *Biochim. biophys. Acta* **401,** 184-195 (1975).
25. Martin, J. B. & Doty, D. M. *Analyt. Chem.* **21,** 965-967 (1949).

Calcium content of mitochondria and endoplasmic reticulum in liver frozen rapidly *in vivo*

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The recognition that the endoplasmic reticulum (ER), rather than the mitochondria, is the main organelle regulating the cytoplasmic Ca^{2+} concentration in non-muscle cells[1,2] supports the notion that an alternative physiological role of mitochondrial Ca transport[3–5] is the modulation of Ca-sensitive mitochondrial enzymes through small (micromolar) fluctuations in the concentration of mitochondrial matrix Ca^{2+} (refs 1, 5–7). The latter mechanism could operate only if the mitochondrial Ca concentration were low, as it is in muscle and retinal rods[8,9], below the levels saturating the regulated enzymes[5]. In contrast, if the ER serves as an intracellular Ca store, its Ca content would be expected to be high. In view of the major metabolic function of the liver, the question of whether hepatic mitochondrial matrix Ca^{2+} regulates metabolism is particularly important, but the range of Ca concentrations reported for isolated liver mitochondria is too wide[10–12] to provide a conclusive answer. Therefore, we have used electron probe X-ray microanalysis (EPMA) to measure the subcellular distribution of Ca in liver snap-frozen *in vivo*, and report here that the endoplasmic reticulum is a major intracellular store of Ca, while the concentration of Ca in mitochondria is low and compatible with the regulation of mitochondrial enzymes.

Figure 1 shows an electron micrograph of an unstained cryosection of rat liver used for electron probe analysis; there is minimal ice-crystal damage, allowing the identification of cell types and intracellular organelles. The Ca concentration in red blood cells within hepatic sinusoids in the same sections used for parenchymal cell measurements, 0.1 ± 0.2 mmol per kg dry wt(± s.e.m.; $n = 34$), is consistent with the micromolar total Ca content of red blood cells[13] and provides an internal estimate

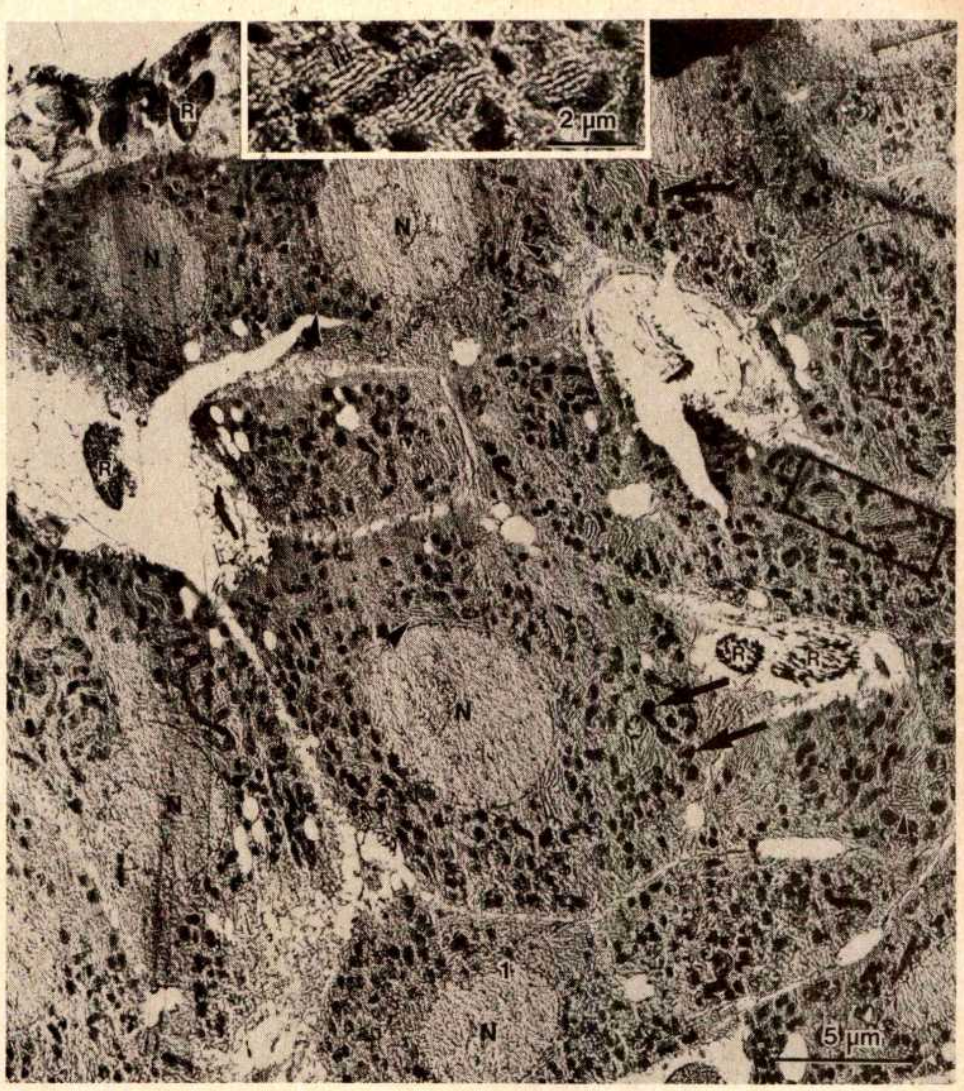


Fig. 1 Electron micrograph of an ultrathin, freeze-dried cryosection obtained from rat liver rapidly clamp-frozen *in situ* with frozen Freon 22. Nuclei (N) and numerous mitochondria (arrows) are indicated, as well as stacks of rough ER (arrowheads). The boxed region is enlarged (inset) to show the stacks of rough ER; some regions show periodic bumps (small arrows) along the membrane which are thought to be ribosomes. The EPMA measurements of the Ca content of ER were obtained from such stacked tubules. Red blood cells (R) are also evident in the liver sinusoids.

Methods. Rats were anaesthetized intraperitoneally with Nembutal (50 mg per kg), an incision made in the abdominal wall over the liver and the rat placed on its abdomen in a cloth hammock above an environmental chamber maintained at 37 °C with high humidity. The liver was exposed gently, without touching its surface, through a slit in the cloth hammock and suspended in the atmosphere of the environmental chamber. The freezing clamp with two opposing aluminium cups filled with solidified Freon 22 was removed from the nitrogen and when the surface of the solid Freon had melted, the door of the environmental chamber was opened and the lobe of the liver clamped rapidly. The temperature of the melted Freon surface is at the melting point of Freon 22 and moulds to the irregular tissue surface, promoting a good contact between the coolant and the tissue. The concept of using melting Freon 'popsicles' originated with Morris Karnovsky (unpublished). The liver was sectioned at −130°C in a cryo-ultramicrotome, then freeze-dried[32].

of the very small errors of the EPMA measurements. The low Ca content of mitochondria and the significantly higher Ca concentration in the ER are shown clearly in the averaged X-ray spectra in Fig. 2. The concentrations of K, Na, Cl, Mg, P and S, measured in the same regions, are given in Table 1. Estimates of the K concentration in fresh liver obtained by other methods are comparable[14].

The mitochondrial calcium concentration was 0.8 ± 0.1 (±s.e.m.) mmol per kg mitochondria ($n = 97$; 29 cells, four rats: two fasted, two fed), which is equivalent to ~1.1 nmol per mg mitochondrial protein (ratio of protein to dry mass = 70%). The ratio of free matrix Ca^{2+} to total mitochondrial Ca, in mitochondria isolated from liver, has been estimated as 7×10^{-4} (ref. 15). Given this value, we can estimate that (for 64% mitochondrial H_2O) the free Ca^{2+} concentration in the mitochondrial matrix of rat liver is ~0.3 μM (95% confidence limit 0.2–0.4 μM), well within the range (0–2 μM) in which mitochondrial dehydrogenases are regulated. If the mitochondrial Ca concentration

Table 1 Subcellular distribution of calcium and other elements in rat liver parenchymal cells (four animals) (mmol per kg dry wt)

	No. of spectra	Na	Mg	P	S	Cl	K	Ca	% Cell volume*	% Total cell Ca
Mitochondria	97	54 ± 3	43 ± 1	360 ± 7	316 ± 4	75 ± 3	314 ± 5	0.8 ± 0.1	20	5
Rough endoplasmic reticulum	24	80 ± 8	76 ± 2	920 ± 21	224 ± 8	94 ± 5	579 ± 17	5.0 ± 0.4	9.4	14–23†
Nuclei	21	77 ± 6	53 ± 2	557 ± 16	245 ± 8	117 ± 6	625 ± 22	0.8 ± 0.4	6	1
Other regions‡	29	75 ± 6	53 ± 2	653 ± 20	241 ± 9	103 ± 5	465 ± 15	4.0 ± 0.4		
Total cell	20	53 ± 4	46 ± 2	564 ± 12	230 ± 9	106 ± 4	479 ± 20	3.4 ± 0.4		

The instrumentation and methods used for EPMA, including calibration, have been published elsewhere[31–34]. To obtain the precision required for these measurements, individual spectra were collected for 400–600 s, until the measurement error of a single spectrum was 1.1 mmol Ca per kg dry wt. Spectra were collected from mitochondria with 75–100-nm diameter probes; from ER with 100-nm or 1,000-nm diameter probes over stacks of ER tubules that included the intervening cytoplasm and, therefore, give an underestimate of the Ca concentration in the ER; from nuclei using probes of diameter 3–10 μm and for total cell measurements, using defocused probes covering whole cell profiles. Values are the mean ± s.e.m.

* From refs 23, 24.

† The lower value is based on the Ca concentration ([Ca]) of the stacks of rough ER, the higher value on the [Ca] measured with 10–20-nm diameter probes over the ER lumen (see text).

‡ Regions in the cell exclusive of nuclei, mitochondria and stacks of rough ER, which, based on known liver structure, would include the smooth ER tubules that were not imaged in the cryosections.

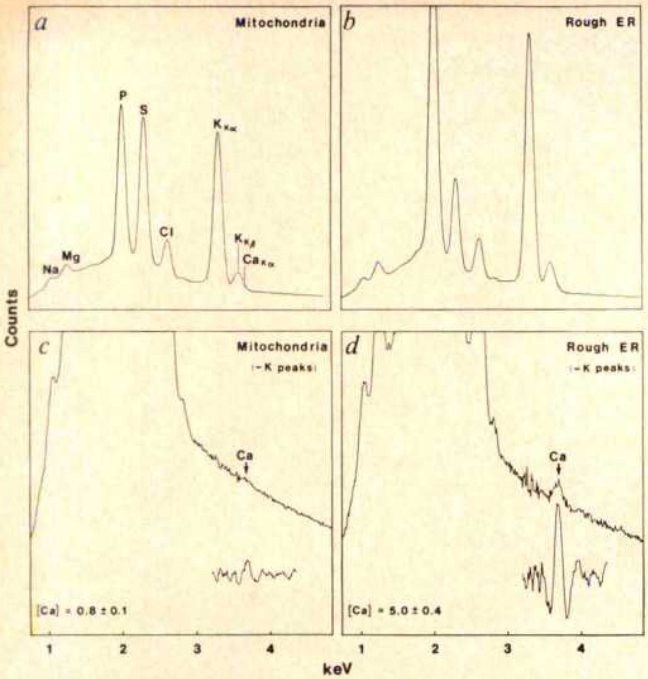


Fig. 2 Averaged X-ray spectra of mitochondria (*a*, *c*) and rough ER (*b*, *d*). The Cu background was subtracted from all spectra by the computer. Peaks representing other elements are indicated. In *c* and *d* (8 × higher gain), the potassium K_α and K_β peaks have also been removed to allow a comparison of the relative sizes of the Ca peaks of mitochondria and ER. The insets show the Ca peaks in the filtered spectra.

were much higher (that is, a total mitochondrial Ca content significantly greater than 2–3 mmol per kg dry wt), the Ca^{2+} regulatory sites on the enzymes would be saturated with Ca^{2+}. Conversely, the mitochondrial Ca content measured by us is insufficient for significant activation of the mitochondrial efflux pathway (apparent $K_m = 9.7\ \mu M$ in isolated liver mitochondria[15]), as would be required for effective mitochondrial regulation of cytoplasmic Ca^{2+} (ref. 5). The reported Ca concentrations of isolated liver mitochondria cover a wide range, depending on the isolation conditions (for review, see ref. 5), with recent values between ~2 and 17 nmol per mg protein[10,12,16]. The lowest values have been found in mitochondria isolated in Ca-free media[10,16], but we note that in smooth muscle[9], incubation in Ca-free solutions reduces the mitochondrial Ca content, measured *in situ*, from 1.6 to 0.1 mmol per kg dry weight.

Our findings also have some implications for the reported release of mitochondrial Ca by agents such as phenylephrine, adrenaline or vasopressin[17]. The Ca content of mitochondria isolated from phenylephrine-treated, isolated hepatocytes in the latter study was higher (5.0 nmol per mg protein) than we found in the untreated animals *in situ*. Therefore, phenylephrine and similar agents, instead of having an *in vivo* effect on mitochondrial Ca, may merely reduce the translocation of Ca into mitochondria during isolation[10], possibly through the depletion of other sources of intracellular Ca, such as the endoplasmic reticulum.

The concentration of Ca in regions of the endoplasmic reticulum was 5.0 ± 0.4 mmol per kg dry wt; this is very much higher than the concentration in mitochondria or in nuclei (Table 1) but may still be an underestimate, as the electron probe diameters were larger than the diameters of the ER tubules (Fig. 1) in the cryosections. The close-packing of the stacks of tubules of rough ER (Figs 1, 3) over which our measurements were made, suggests that the Ca concentration may have been underestimated by a factor of 2, and that the true Ca content of the rough ER is perhaps 8–10 mmol per kg dry weight. This value is still much higher than the Ca content (~2 nmol per mg protein) of microsomes isolated from liver[11], but is comparable to the steady-state Ca content of 10 nmol Ca per mg protein

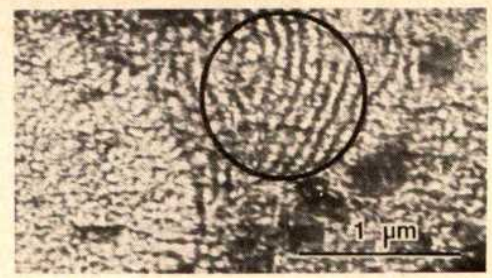


Fig. 3 High-magnification view of a portion of a parenchymal liver cell in an ultrathin cryosection, showing a stacked array of rough endoplasmic reticulum. The encircled region indicates the area that was irradiated with the electron beam during electron probe microanalysis, and which contained the following concentrations (± s.d.) of calcium, magnesium and phosphorus: 4.2 ± 0.5, 87.0 ± 1.5 and 995 ± 4.6 mmol per kg dry wt, respectively.

in microsomes equilibrated with 70 nM free Ca^{2+} (ref. 18).

The high phosphorus (920 ± 21 mmol per kg dry wt) and magnesium (76.1 ± 1.9 mmol per kg dry wt) concentrations in the stacks of rough ER reflect the presence of membrane phospholipids and ribosomes. When probes from a field emission gun were focused (8–20 nm diameter) on ribosomes attached to the stacked ER, the P and Mg contents measured (respectively, 1,129 ± 77 and 89.9 ± 3.5 mmol per kg dry wt, $n = 4$) were even higher, confirming that ribosomes[19,20] make a large contribution to the high P and Mg concentrations in the stacked rough ER. The Ca concentration in the ribosomes was 0.1 ± 0.6 mmol per kg dry wt, and in the lumen of ER tubules (measured with similar small (8–20-nm) probes) it was 8.2 ± 1.9 mmol per kg dry wt ($n = 4$), consistent with the estimate based on measurements made with defocused probes over stacks of rough ER.

The 'cytoplasmic' regions (labelled 'other' in Table 1) that excluded visible organelles (mitochondria, nuclei, stacks of rough ER) had a relatively high Ca content but, based on known liver morphology, they probably also included tubules of smooth and/or rough ER not imaged in unstained cryosections. Therefore, some of the Ca measured in these cytoplasmic regions may be present in the lumen of non-visualized ER, as well as bound to cytoplasmic and membranous proteins such as calmodulin, calelectrin, etc.[21,22]. The Ca content of nuclei was 0.8 ± 0.4 mmol Ca per mg protein ($n = 21$), providing an upper limit for the Ca that could be bound to small, diffusible cytoplasmic proteins capable of passing through nuclear pores. We were unable to determine the Ca content of individual tubules of smooth ER that were not visualized in the unstained cryosections. EPMA measures total (free and bound) Ca, and our measurements are not directly comparable with values of free and exchangeable Ca measured using other methods.

We determined the subcellular distribution of total liver cell Ca by combining our results with stereological measurements[23,24] of the fractional volume occupied by various organelles (Table 1); the Ca content of the mitochondria (20% of the cell volume) and of the nucleus (6% of the cell volume) accounts for only ~5% and 1%, respectively, of the 3.4 mmol per kg dry wt total cell Ca. The Ca content of whole fresh rat liver, including extracellular Ca, is reported to be 4.1 mmol per kg dry wt[5]. The minimal fraction of cell Ca in the rough ER, based on a 9.4% volume and on the Ca measurements obtained with defocused probes over the stacks of rough ER, is 14%; this value increases to 23% if the Ca content of the lumen of the rough ER is included. Considering the total cell volume of the ER (15% = 9.4% rough ER and 5.9% smooth ER) and assuming that the Ca concentration in the smooth ER is the same as that in the rough ER, the fraction of the total cell Ca present in the ER could be 23–27%.

Thus, our results support the view[1,6,7,26] that mitochondrial enzymes may be regulated by matrix free Ca^{2+}, and extend it to a metabolically active, intact, blood-perfused organ. The mitochondrial Ca content is far below the concentration[5] at

which the mitochondrial Ca efflux pathway is active and mitochondria could effectively regulate cytoplasmic free Ca^{2+}. The high Ca content of the ER is consistent with the view that this is the source of Ca released by inositol trisphosphate[27-30] and other agents. Our results also show that it is possible to freeze some cells in a near physiological condition *in vivo*, and make direct measurements with EPMA of the composition of organelles at high spatial resolution and sensitivity[31,32].

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1. Somlyo, A. P. *Nature* **309**, 516-517 (1984).
2. Martonosi, A. N. in *Muscle and Nonmuscle Motility* Vol. 1 (ed. Stracher, A.) 233 (Academic, New York, 1983).
3. Carafoli, E. & Crompton, M. *Ann. N.Y. Acad. Sci.* **307**, 269-284 (1978).
4. Scarpa, A. in *Membrane Transort in Biology II* (eds Giebisch, G., Tosteson, D. C. & Ussing, H. H.) 263-355 (Springer, New York, 1979).
5. Hansford, R. G. *Rev. Physiol. biochem. Physiol.* (in the press).
6. Denton, R. M. & McCormack, J. G. *FEBS Lett.* **119**, 1-8 (1980).
7. Carafoli, E. in *Exercise Bioenergetics and Gas Exchange* (eds Cerretelli, P. & Whipp, B. J.) 3-12 (North-Holland, Amsterdam, 1980).
8. Somlyo, A. P. & Walz, B. *J. Physiol., Lond.* **358**, 183-195 (1985).
9. Bond, M., Shuman, H., Somlyo, A. V. & Somlyo, A. P. *J. Physiol., Lond.* **357**, 185-201 (1984).
10. Reinhart, P. H., van de Pol, E., Taylor, W. M. & Bygrave, F. L. *Biochem. J.* **218**, 415-420 (1984).
11. Becker, G. L., Fiskum, G. & Lehninger, A. L. *J. biol. Chem.* **255**, 9009-9012 (1980).
12. Joseph, S. K., Coll, K. E., Cooper, R. H., Marks, J. S. & Williamson, J. R. *J. biol. Chem.* **258**, 731-741 (1983).
13. Bookchin, R. M. & Lew, V. L. *Nature* **284**, 561-563 (1980).
14. Howard, R. B., Lee, J. C. & Pesch, L. A. *J. Cell Biol.* **57**, 642-658 (1973).
15. Coll, K. E., Joseph, S. K., Corkey, B. E. & Williamson, J. R. *J. biol. Chem.* **257**, 8696-8704 (1982).
16. Blackmore, P. F., Dehaye, J.-P. & Exton, J. H. *J. biol. Chem.* **254**, 6945-6950 (1979).
17. Babcock, D. F., Chen, J.-L. J., Yip, B. P. & Lardy, H. A. *J. biol. Chem.* **254**, 2117-2120 (1979).
18. Dawson, A. P. *Biochem. J.* **206**, 73-79 (1982).
19. Hamilton, M. G. & Peterman, M. L. *J. biol. Chem.* **234**, 1441-1446 (1959).
20. Siekevitz, P. & Palade, G. E. *J. biophys. biochem. Cytol.* **7**, 631-644 (1960).
21. Sudhof, T. C. *Biochem. biophys. Res. Commun.* **123**, 100-107 (1984).
22. Waisman, D. W., Smallwood, J., Lafreniere, D. & Rasmussen, H. *Biochem. biophys. Res. Commun.* **119**, 440-446 (1984).
23. Weibel, E. R., Staubli, W., Guagi, R. & Hess, F. A. *J. Cell Biol.* **42**, 68-91 (1969).
24. Loud, A. V. *J. Cell Biol.* **37**, 27-46 (1968).
25. van Rossum, G. D. V. *J. gen. Physiol.* **55**, 18-31 (1970).
26. Hansford, R. G. & Castro, F. *J. Bioenerg, Biomembranes* **14**, 361-376 (1982).
27. Berridge, M. J. *Biochem. J.* **212**, 849-858 (1983).
28. Burgess, G. M. *et al. Nature* **309**, 63-66 (1984).
29. Dawson, A. P. & Irvine, R. F. *Biochem. biophys. Res. Commun.* **120**, 858-864 (1984).
30. Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. *J. biol. Chem.* **259**, 3077-3081 (1984).
31. Hall, T. A. & Gupta, B. L. *Q. Rev. Biophys.* **16**, 279-330 (1983).
32. Somlyo, A. V., Gonzalez-Serratos, H., Shuman, H., McClellan, G. & Somlyo, A. P. *J. Cell Biol.* **90**, 577-594 (1981).
33. Shuman, H., Somlyo, A. V. & Somlyo, A. P. *Ultramicroscopy* **1**, 317-339 (1976).
34. Kitazawa, T., Shuman, H. & Somlyo, A. P. *Ultramicroscopy* **11**, 251-262 (1983).

Identification of the human analogue of a regulator that induces differentiation in murine leukaemic cells

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We have recently purified murine granulocyte colony-stimulating factor (G-CSF)[1], a regulatory glycoprotein which stimulates granulocyte colony formation from committed murine precursor cells in semi-solid agar cultures[2]. G-CSF is one of a family of colony-stimulating factors that regulate the growth and differentiation of granulocytes and macrophages[3-5]. While the other murine CSFs (granulocyte–macrophage (GM)-CSF, macrophage (M)-CSF and multi-CSF) show little or no differentiation-inducing activity on murine myelomonocytic leukaemia cell lines, G-CSF (or MGI-2[6]) is able to induce the production of terminally differentiated cells from WEHI-3B and other myeloid leukaemia cell lines[6-8]. More importantly, G-CSF-containing materials suppress the self-renewal of myeloid leukaemia stem cells *in vitro*[8] and the leukaemogenicity of treated myeloid leukaemic cells *in vivo*[9-13]. It is important to identify the human analogue of murine G-CSF so that its effectiveness on human myeloid leukaemia cells can be assessed. Here we show that an analogue of G-CSF does exist among the CSFs produced by human cells and that the murine and human molecules show almost complete biological and receptor-binding cross-reactivities to normal and leukaemic murine or human cells. The human G-CSF analogue is identified as a species of CSF that we have previously described as CSF-β (ref. 14).

Two CSFs in human placental conditioned medium that are active on human granulocyte and macrophage progenitor cells have been separated on the basis of differences in hydrophobicity[14]; the earlier-eluting species (CSF-α) stimulated the formation of neutrophilic granulocyte, macrophage and eosinophilic granulocyte colonies with colony formation peaking by day 14 of culture, while the later-eluting species (CSF-β) stimulated almost exclusively the formation of neutrophilic granulocyte colonies peaking by day 5-7 of culture. We have confirmed the existence of these two species of human CSF by using medium conditioned by the human bladder cell line 5637 (Fig. 1), as have others using medium conditioned by the GCT cell line[15]. After fractionation on phenyl-Sepharose (Fig. 1), CSF-β fractions which were active on human bone marrow cells also stimulated granulocyte colony formation by murine marrow cells and induced differentiation in murine myelomonocytic leukaemia WEHI-3B D^+ cells, whereas CSF-α fractions had no such effects. The low frequency, small size and maturity of the murine granulocytic colonies stimulated by CSF-β were typical of colonies stimulated by G-CSF[2].

In agar cultures of human bone marrow cells, pure G-CSF stimulated granulocytic colony and cluster formation comparable in number, size and rate of development to those stimulated by CSF-β. To minimize possible indirect effects of CSF-producing cells in unfractionated human marrow cultures, we compared the activities of G-CSF and CSF-β in cultures of human marrow cell fractions highly enriched for promyelocytes and myelocytes by fluorescence-activated cell sorting (FACS)[16]. The dose-response curves for proliferation in cultures of these cells stimulated by partially purified CSF-α, CSF-β and pure G-CSF were almost identical in shape and, in particular, CSF-β and G-CSF stimulated the development of equal numbers of clones (Fig. 2*a*). CSF-β and G-CSF also showed similar dose-response curves for inducing differentiation in WEHI-3B D^+ colonies, while CSF-α was inactive in this assay (Fig. 2*b*). Interestingly, the dose-response curves for inducing differentiation of WEHI-3B D^+ colonies were shifted eightfold to the left for both CSF-β and G-CSF compared with the dose-response curves for human progenitor cells (note the different dilutions used in Fig. 2*a*, *b*).

^{125}I-labelled G-CSF demonstrates specific, saturable binding to WEHI-3B D^+ leukaemic cells and normal murine bone marrow cells[17]. ^{125}I-G-CSF also bound specifically to normal human bone marrow and blood cells as well as a variety of fresh human myeloid leukaemic cells (Table 1). The binding of ^{125}I-G-CSF to human leukaemias paralleled its specificity of action on normal target cells. Cells from all acute myeloblastic leukaemias, chronic myeloid leukaemias, acute promyelocytic leukaemias and pre-leukaemias showed higher levels of binding than those observed for lymphocytic leukaemia cells. Acute myelomonocytic leukaemias showed relatively little binding of ^{125}I-G-CSF compared with the granulocytic leukaemias; this granulocytic specificity of the binding exhibited by ^{125}I-G-CSF was confirmed by autoradiography of human bone marrow cells (Fig. 3) which showed that cells of the granulocytic cell lineages were specifically labelled (promyelocytes more intensely labelled than polymorphs) while lymphoid and erythroid cells were not. For comparison, Fig. 3 shows specific labelling of acute promyelocytic leukaemic blast cells.

The binding of ^{125}I-G-CSF to WEHI-38 D^+ cells is inhibited in a dose-dependent manner by unlabelled G-CSF but not by

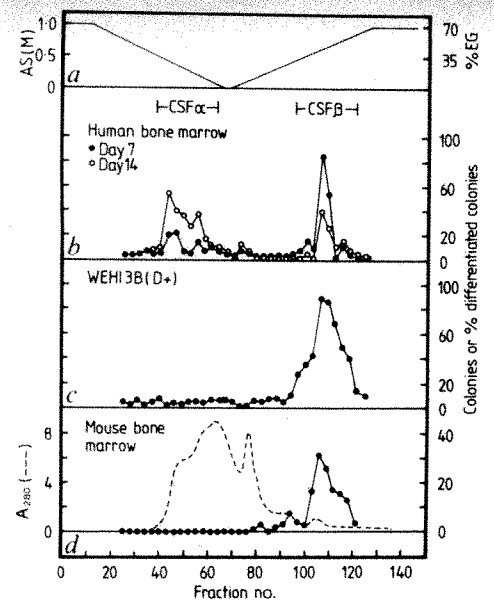


Fig. 1 Separation of human bladder cell line (5637) conditioned medium on phenyl-Sepharose CL-4B. The bladder cell line[21,22] was incubated at 1×10^6 cells ml^{-1} in DMEM containing 5% FCS at 37 °C in 10% CO_2 in air for 7 days. The conditioned medium was centrifuged at 10,000*g* for 30 min, made 1 M in ammonium sulphate and applied to a column of phenyl-Sepharose CL-4B (2.6 × 40 cm) equilibrated in 1 M ammonium sulphate/0.1 M sodium phosphate buffer *p*H 6.0 containing Tween 20 (0.02%) and sodium azide (0.02%) (start buffer). The column was eluted with a 200-ml linear gradient from start buffer to distilled water, followed by a 200-ml linear gradient from distilled water to 70% ethylene glycol (*a*) at a flow rate of 20 ml h^{-1}, and 3.5-ml fractions were collected. The fractions were exchanged into phosphate-buffered (20 mM, *p*H 7.4) saline (0.15 M) containing Tween 20 (0.02%) by passage through Sephadex G-25M and sterilized by passage through Millipore 0.45-μm filters. Aliquots of each fraction were then assayed for their ability to stimulate colony formation of normal bone marrow cells from human rib (100,000 cells per plate), colonies being scored on days 7 and 14 of culture (*b*); their ability to induce differentiation in colonies formed by murine myelomonocytic leukaemia cells (WEHI-3B D^+) (300 cells per plate, ~180 colonies) after 7 days of culture (*c*); and their ability to induce colony formation by normal murine C57BL/6 bone marrow cells (75,000 cells per plate) after 7 days of culture (*d*). In all cases cultures were prepared in 35-mm Petri dishes containing 0.3% agar, DMEM and 20% FCS and 10% by volume of fraction to be assayed, and were incubated at 37 °C in 10% CO_2 in air in a fully humidified incubator. Colonies comprising >50 cells were scored at ×35 magnification under a dissecting microscope and differentiated WEHI-3B D^+ colonies were enumerated by their characteristic dispersed morphology. The absorbance of the fractions at 280 nm (dashed line) is also shown in *d*. AS, ammonium sulphate concentration; % EG, percentage by volume of ethylene glycol in water.

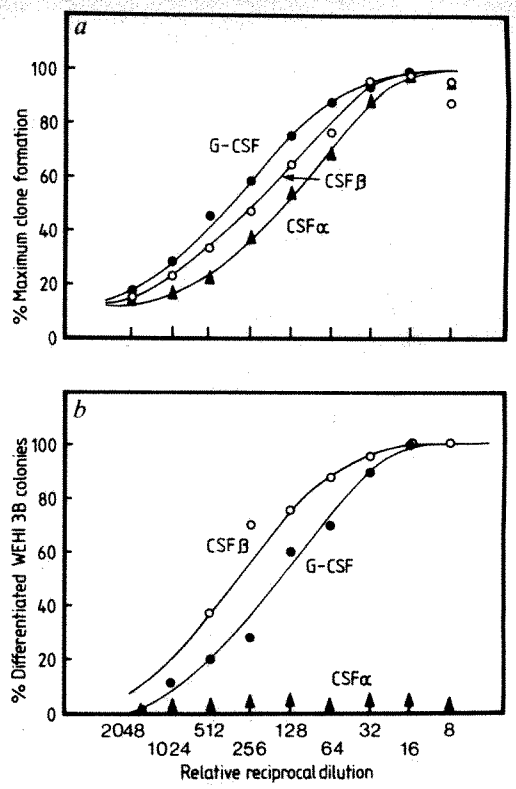


Fig. 2 Ability of human and murine colony-stimulating factors (CSFs) to stimulate clonal proliferation by human cells (*a*) or to induce differentiation in a murine leukaemic cell line (*b*). Human CSF-α and CSF-β preparations were from bladder cell line-conditioned medium separated by phenyl-Sepharose CL-4B chromatography (pooled as shown in Fig. 1), and murine G-CSF was pure material from mouse lung-conditioned medium, purified as described elsewhere[1]. The human promyelocyte-rich cell fraction (*a*) was obtained from normal human bone marrow cells after fractionation by FACS using a monoclonal antibody raised against human neutrophils as described elsewhere[16]. This fraction contained 35% promyelocytes and myelocytes with no other cells capable of proliferation. Each CSF was titrated in serial twofold dilutions for its ability to stimulate total clone formation (>2 cells) in agar cultures of 2,000 enriched cells as described in Fig. 1 legend, except that clones were scored on day 5 of culture. Maximum clone numbers were 175 for CSF-β or G-CSF and 150 for CSF-α. The distribution of clone sizes was almost identical for CSF-β and G-CSF (2–21 cells) and clones were larger than for CSF-α (2–9 cells). The same pools of CSFs were assayed for their ability to induce a differentiated morphology in murine WEHI-3B D^+ colonies after 7 days of culture (*b*). Note that the dilutions of samples in *b* were eightfold diluted relative to the dilution of samples in *a* in order to compare more easily the span of the titration curves in the two assay systems.

M-CSF, GM-CSF, multi-CSF or any of several unrelated growth factors[17]. Figure 4*c* shows that human CSF-β inhibited ^{125}I-G-CSF binding to WEHI-3B D^+ cells to the same extent as unlabelled G-CSF and that the dose-response relationship was indistinguishable from that for G-CSF when the data were standardized to the number of units added, based on the human colony-forming assay of Fig. 2. In contrast, human CSF-α and murine GM-CSF showed no inhibition of binding over the same dose range. Marrow cells of light density from patients with untreated chronic myeloid leukaemia or untreated acute myeloid leukaemia (with >90% leukaemic blast cells) gave binding data that were almost identical to those seen for murine WEHI-3B D^+ cells (Fig. 4*a*, *b*). Moreover, the dose-response relationships for inhibition of ^{125}I-G-CSF binding by unlabelled G-CSF or CSF-β were identical and occurred over the same concentration range as the inhibition of binding to WEHI-3B D^+ cells while, again, CSF-α and GM-CSF did not inhibit binding. Figure 4*d* shows that the binding of ^{125}I-G-CSF to BALB/c bone marrow cells was competed for equally by unlabelled G-CSF and CSF-β and that CSF-α, like GM-CSF, could inhibit only 30% of the specific binding sites; the latter effect appears to result from down-regulation of G-CSF receptors rather than receptor cross-reactivity (N.A.N. and D.M., unpublished).

Saturation binding and Scatchard analyses were performed for the binding of ^{125}I-G-CSF to human acute myeloid leukaemic bone marrow cells (>95% leukaemic blast cells) and murine leukaemic WEHI-3B D^+ cells (Fig. 5). Because we have noted previously[17] that Scatchard analysis of binding data for WEHI-3B D^+ cells becomes nonlinear at low levels of bound ^{125}I-G-CSF, the dissociation constants were determined from the limiting slopes of the Scatchard curves; these gave a dissociation constant of 102 pmol for binding to WEHI-3B D^+ cells and 98 pmol for binding to the acute myeloid leukaemic blast cells. The number of binding sites per cell, estimated from the intercepts on the abscissa, were 300 and 480 for WEHI-3B D^+ and human acute myeloid leukaemic cells, respectively.

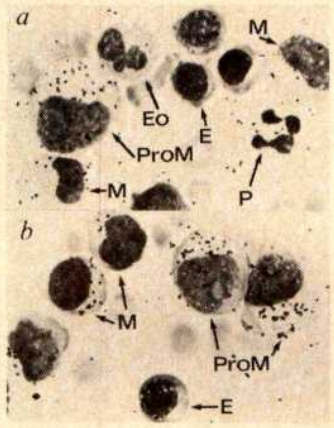


Fig. 3 Autoradiographs of the binding of ^{125}I-G-CSF to: *a*, normal human marrow; and *b*, acute promyelocytic leukaemic marrow. In *a*, note the heavily labelled promyelocyte (ProM) and labelled myelocytes (M) and polymorph (P) but unlabelled eosinophil (Eo) and erythroid cell (E). In *b*, note the labelled promyelocytes and myelocytes but unlabelled erythroid cell. Exposure time 1 month.

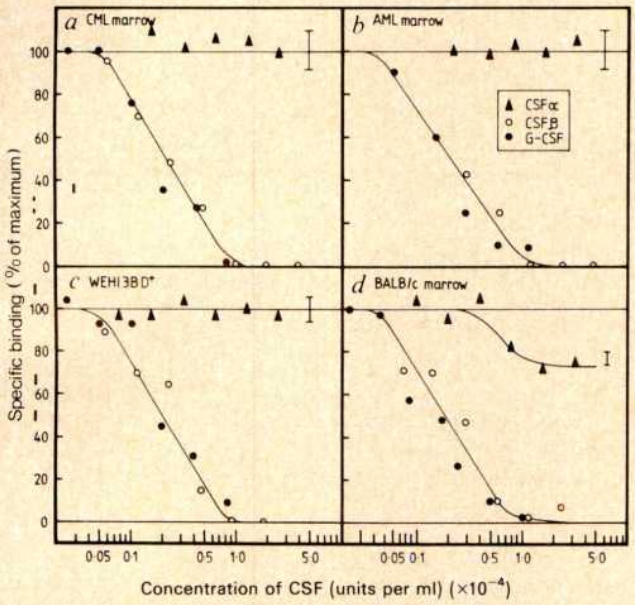


Fig. 4 Ability of human and murine colony-stimulating factors to compete for the binding of murine G-CSF to human and murine cells. Human CSF-α and CSF-β pools and murine G-CSF were obtained as described in Fig. 2 legend and units (U) standardized in the assay on human promyelocyte-enriched cells, as described in Fig. 2 legend (50 U being that amount giving 50% of a maximal clone response). ○, Unlabelled CSF-β; ●, unlabelled G-CSF; and ▲, unlabelled CSF-α. Iodinated G-CSF (prepared as described elsewhere[17] (80,000–200,000 c.p.m., specific activity 150,000–300,000 c.p.m. ng^{-1}) and increasing amounts of unlabelled G-CSF, CSF-α or CSF-β as shown were added to 5×10^6 human chronic myeloid leukaemia (CML) bone marrow cells (density <1.077 g cm^{-3}) (*a*), 5×10^6 human acute myeloid leukaemia (AML) bone marrow cells (density <1.077 g cm^{-3}) (*b*), 2×10^6 WEHI-3B D$^+$ cells (*c*) or 5×10^6 BALB/c murine bone marrow cells (*d*) in DMEM/10% FCS (90 μl final volume) and incubated at 37 °C in 10% CO_2 in air for 1 h. Cells were then centrifuged (300*g*, 5 min) through 200 μl ice-cold FCS, the cell pellet was collected by cutting off the end of the tube, and this was counted in a γ-counter at 76% efficiency. Maximum (100%) specific binding was that in the absence of competitors (mean of duplicate tubes) and 0% specific binding was that in the presence of a 50-fold excess of unlabelled G-CSF (mean of duplicate tubes). The bar on the right-hand side of each panel represents the per cent specific binding seen in the presence of 100,000 U of murine GM-CSF (range for triplicate or more tubes).

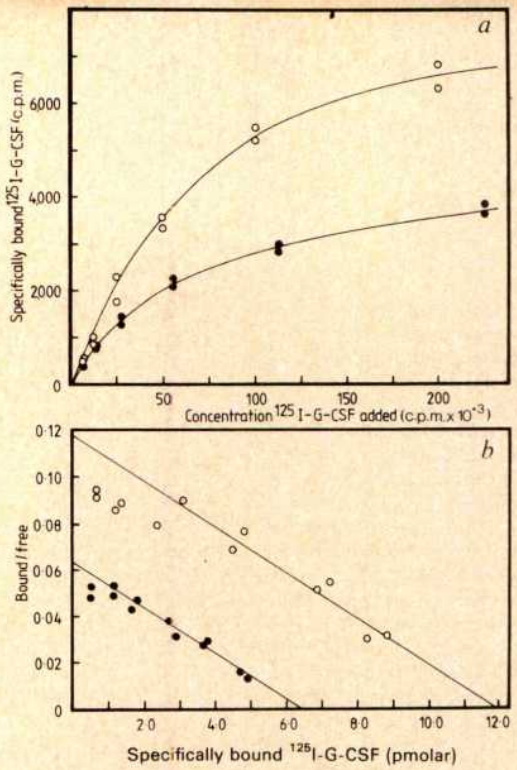


Fig. 5 Saturation analysis of binding of ^{125}I-G-CSF to human AML cells (○) and murine myelomonocytic leukaemic cell line WEHI-3B D$^+$ (●). Specific binding was determined as total binding − nonspecific binding; *a* shows the results of duplicate determinations. *b*, The same data converted to Scatchard plots (specific c.p.m. bound/c.p.m. in the supernatants versus the concentration of ^{125}I-G-CSF specifically bound to cells).

Methods. A freshly obtained bone marrow aspirate from a patient with acute myeloid leukaemia classified as M1 was resuspended in DMEM/FCS, layered over Ficoll/Paque (Pharmacia) of density 1.077 g cm^{-3} and centrifuged at 700*g* for 20 min. The interface cells (>95% leuukaemic blast cells by morphology of stained preparations) were resuspended in DMEM/FCS and passed through a loosely packed, sterile cotton wool plug to remove fat particles. AML cells (2.5×10^6) or WEHI-3B D$^+$ cells (2.0×10^6) were incubated with increasing concentrations of ^{125}I-G-CSF as shown, with or without an excess of unlabelled G-CSF (250 ng ml^{-1}) in DMEM/10% FCS (total volume 170 and 160 μl, respectively) at 37 °C for 1 h in a fully humidified incubator in 10% CO_2 in air. Tubes were then placed on ice, the cells resuspended and layered over 200 μl cold FCS in 0.5 ml flexible plastic centrifuge tubes and centrifuged at 300*g* for 5 min. The tubes were then cut with a scalpel blade a few millimetres above the cell pellet and the pellet and supernatants counted separately in a γ-counter. Nonspecific binding (that in the presence of excess unlabelled G-CSF) was linear with respect to the amount of ^{125}I-G-CSF added and was maximally 15% of the specific binding in these experiments.

Most murine haematopoietic growth factors are inactive on human cells but the species cross-reactivity of murine G-CSF and human CSF-β demonstrated here appears to be quite unambiguous. Human CSF-β and murine G-CSF are both hydrophobic molecules of similar size (relative molecular mass 30,000 and 25,000 respectively) and both are able to stimulate granulocyte colony formation by cells from both species with similar dose–response relationships and similar numbers of target cells responsive to either stimulus. That this represents direct cross-reactivity was indicated by the demonstration of specific binding sites for ^{125}I-G-CSF on human and murine granulocytic cells and the equal abilities of G-CSF and CSF-β to inhibit this binding.

Both molecules also induced differentiation in murine WEHI-3B cells and showed specific binding sites on human myeloid leukaemias. Moreover, CSF-β has been shown to induce differentiation of cells from the human promyelocytic leukaemia cell

Table 1 Specific binding of murine ^{125}I-G-CSF to human normal and leukaemic cells

Type of patient (F.A.B. classification)	Cell source	No. of patients	Specific binding (% of WEHI-3B D$^+$) Mean (±s.d.)	Range
Normal	Bone marrow	5	34±16	17-55
	Blood buffy coat	4	12±11	1-27
	Purified blood neutrophils	5	86±29	45-114
Preleukaemia*	Bone marrow	4	103±55	62-184
Acute promyelocytic	Bone marrow	2		163, 82
leukaemia (M3)†	Blood	1		19
Chronic myeloid	Bone marrow	5	66±26	27-97
leukaemia‡	Blood	3	56±6	52-63
Acute myeloblastic	Bone marrow	9	56±41	18-140
leukaemia (M1+M2)†	Blood	9	30±17	12-56
Acute myelomonocytic	Bone marrow	2		4, 7
leukaemia (M4)†	Blood	2		5, 13
Acute lymphoblastic	Bone marrow	3	4±4	1-8
leukaemia†	Blood	1		<1
Chronic lymphocytic leukaemia§	Blood	3	4±6	−1-10

Leukaemic cells of density $<1.077\ g\ cm^{-3}$ were prepared by centrifugation over Ficoll/Paque (Pharmacia) at 700*g* for 20 min. Normal blood cells comprised unfractionated buffy coat cells. Normal granulocytes were purified from blood by density centrifugation in hypertonic metrizamide gradients[20] and were 95-100% pure. Cells, usually 5×10^6 per incubation, were incubated in duplicate with 90,000-300,000 c.p.m. of ^{125}I-G-CSF with or without a 20-fold excess of unlabelled G-CSF in Dulbecco's modified Eagle's medium (DMEM)/ fetal calf serum (FCS) (70-130 μl total volume) at 37 °C for 1 h. Specific binding (total binding − binding in the presence of unlabelled G-CSF) is expressed as a percentage of the specific binding to an equivalent number of WEHI-3B D$^+$ cells using the same preparation of ^{124}I-G-CSF on the same day.

* Including a patient with refractory anaemia with excess blast cells.

† Acute leukaemic patients were newly diagnosed or in relapse and their density-fractionated cells contained >80% leukaemic blasts.

‡ Cells taken at diagnosis before any treatment.

§ All showed >90% leukaemic lymphocytes.

line HL60 (ref. 18). The frequency of binding sites on leukaemic cells was similar to that on the equivalent normal cells and the apparent binding affinity for ^{125}I-G-CSF appeared to be approximately the same on all the cell types studied. Finally, other studies have shown that G-CSF is able to stimulate various functional activities of post-mitotic neutrophil granulocytes of mouse and man[19].

The strong conservation of G-CSF/CSF-β and their receptors through evolution signals their importance for the maintenance of granulocytic cells in the animal. The conservation of receptors on human granulocytes and myeloid leukaemias indicates that G-CSF and its human analogue, CSF-β, merit further study as regards their possible therapeutic usefulness.

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1. Nicola, N. A., Metcalf, D., Matsumoto, M. & Johnson, G. R. *J. biol. Chem.* **258**, 9017-9021 (1983).
2. Metcalf, D. & Nicola, N. A. *J. cell. Physiol.* **116**, 198-206 (1983).
3. Metcalf, D. *Hemopoietic Colonies* (Springer, Berlin, 1977).
4. Nicola, N. A. & Vadas, M. *Immun. Today* **5**, 76-80 (1984).
5. Burgess, A. W. & Metcalf, D. *Blood* **56**, 947-958 (1980).
6. Lotem, J., Lipton, J. H. & Sachs, L. *Int. J. Cancer* **25**, 763-771 (1980).
7. Burgess, A. W. & Metcalf, D. *Int. J. Cancer* **26**, 647-654 (1980).
8. Metcalf, D. *Int. J. Cancer* **25**, 225-233 (1980).
9. Ichikawa, Y. *J. cell. Physiol.* **76**, 175-184 (1970).
10. Fibach, E. & Sachs, L. *J. cell. Physiol.* **86**, 221-230 (1975).
11. Honma, J., Kasukabe, T., Okabe, J. & Hozumi, M. *Cancer Res.* **39**, 3167-3171 (1979).
12. Metcalf, D. *J. natn. Cancer Inst. Monogr.* **60**, 123-131 (1982).
13. Lotem, J. & Sachs, L. *Int. J. Cancer* **28**, 375-386 (1981).
14. Nicola, N. A., Metcalf, D., Johnson, G. R. & Burgess, A. W. *Blood* **54**, 614-627 (1979).
15. Abboud, C. N., Brennan, J. K., Barlow, G. N. & Lichtman, M. A. *Blood* **58**, 1148-1154 (1981).
16. Begley, C. G., Lopez, A. F., Vadas, M. A. & Metcalf, D. *Blood* (in the press).
17. Nicola, N. A. & Metcalf, D. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3765-3770 (1984).
18. Metcalf, D. *Leuk. Res.* **7**, 117-132 (1983).
19. Lopez, A. F. *et al. J. Immun.* **131**, 2983-2988 (1983).
20. Vadas, M. A., David, J. R., Butterworth, A. E., Pisani, N. T. & Songok, T. A. *J. Immun.* **122**, 1228-1236 (1979).
21. Fogh, J. *J. natn. Cancer Inst. Monogr.* **49**, 5-9 (1978).
22. Svet-Moldavsky, G. J. *et al. Expl Hemat.* **8** (Suppl. 7), 76 (1983).

Hybrid antibodies can target sites for attack by T cells

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It would be advantageous in the case of certain diseases to be able to focus a strong T-cell response at a chosen target, for example, in treating cancer or infections that have escaped the normal host response. At present, it seems inconceivable that we could use antigen-specific lines or clones of effector T cells for this purpose because of complications due to the major histocompatibility restriction of T-cell specificity and the problem of rejection of transplanted effector cells. Here we describe a novel technology which combines the power of T lymphocytes in eliminating unwanted cells and causing beneficial inflammatory reactions with the great advantages of monoclonal antibodies (their specificity and availability). We show that heteroconjugates of monoclonal antibodies (referred to hereafter as hybrid antibodies), in which one of the component binding sites is anti-T-cell receptor and the other component binding site is directed against any chosen target antigen, can focus T cells to act at the targeted site. Monoclonal antibodies directed against the T-cell receptor, such as the anti-allotype used here, are mitogenic for resting T cells and can be used to induce effector T cells carrying the T-cell receptor determinant which can then be directed against the target by a hybrid antibody.

Monoclonal antibodies directed against determinants of the T-cell receptor complex of cytotoxic T lymphocytes (CTL) or T-helper cells are able to mimic the effect of antigen in many ways. For example, anti-idiotypes in an appropriate form can

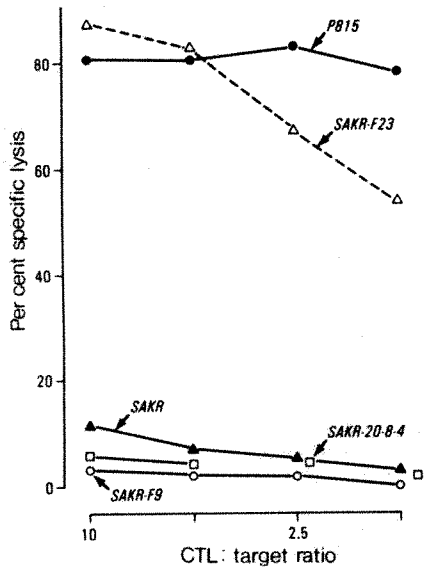


Fig. 1 Anti-receptor allotype monoclonal antibody F23.1 coupled to the target cell surface renders the cell susceptible to lysis by an allotype-positive CTL clone, OE4. F23.1 (IgG2a)[8], F9 (IgG1) monoclonal antibody specific for an idiotypic determinant on CTL clone G4 (ref. 3) and the anti-H-2^b monoclonal antibody 20-8-4 (IgG2a)[15], produced in C3H mice, were purified from ascites fluids on protein A-Sepharose. Antibodies were reacted with the heterobifunctional crosslinker SPDP (Sigma)[16]; a threefold molar excess of SPDP was used to introduce two to three groups per 7S antibody. The modified antibody was then reacted with S.AKR lymphoma cells (H-2^k, Thy-1.1) which had been pretreated with 0.5 mM dithiothreitol to introduce free sulphydryl groups at the cell surface[17,18]. After washing, 1×10^4 pre-labelled (^{51}Cr-labelled sodium chromate) target cells were incubated at 37 °C with twofold serial dilutions of the CTL clone OE4 at 37 °C for 4 h (ref. 3). Per cent specific lysis of these target cells and of P815 (DBA/2, H-2^d) targets was calculated as described elsewhere[3]. The CTL clone OE4 was isolated by limiting dilution from a C57BL/6 (H-2^b) anti-DBA/2 (H-2^d) mixed lymphocyte culture and is maintained by weekly subculture with irradiated DBA/2 spleen cells plus IL-2. OE4 reacts with the anti-allotypic antibody F23.1 and with the anti-H-2^b antibody 20-8-4 but not with the anti-idiotypic F9 antibody. Target cells were: P815 (●), S.AKR (▲), S.AKR coupled to F23.1 (△), S.AKR coupled to F9 (○) and S.AKR coupled to 20-8-4 (□).

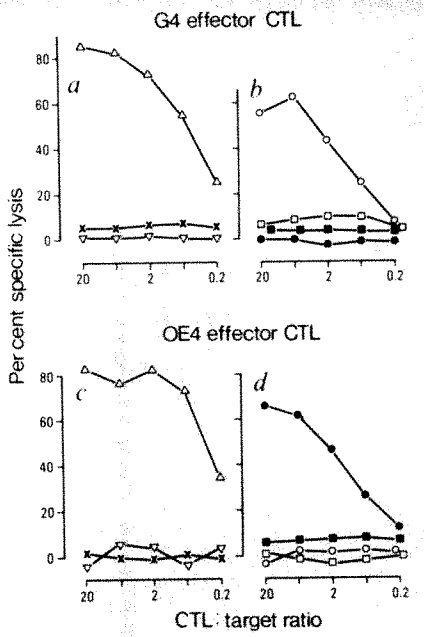


Fig. 2 Heteroconjugates of monoclonal antibodies (hybrid antibodies) can specifically target cells for CTL-mediated lysis. Effector CTL clones were: *a* and *b*, G4 [BALB.B (H-2^b) anti-BALB/c (H-2^d)], which is positive for the idiotypic determinant recognized by F9 (ref. 3), and unreactive with F23; *c* and *d*, OE4 [C57BL/6 (H-2^b) anti-DBA/2 (H-2^d)] CTL clone which is F23$^+$, F9$^-$. Target cells were: P815 (H-2^d, △), S.AKR (H-2^k, ▽), EL4 (H-2^b, ×), S.AKR incubated with 19E12/F23 (●), S.AKR incubated with 19E12/F9 (○), EL4 incubated with 19E12/F23 (■), and EL4 incubated with 19E12/F9 (□).
Methods. The monoclonal alloantibody 19E12 (ref. 11) (IgG2a) specific for the Thy-1.1 alloantigen was purified from ascites fluid on protein A-Sepharose, modified with SPDP[16] and coupled to either F23.1 or F9 monoclonal antibody according to the manufacturer's handbook (Pharmacia). The hybrid antibodies were used to coat ^{51}Cr-labelled EL4 (H-2^b, Thy-1.2) and S.AKR (H-2^k, Thy-1.1) lymphoma cells by incubation at 37 °C for 20 min followed by washing. These target cells plus uncoated ^{51}Cr-labelled S.AKR, EL4 and P815 (H-2^d) targets were assayed for lysis by serial threefold dilutions of CTL at 37 °C for 4 h.

stimulate idiotype-positive T-cell clones to release soluble factors such as interleukin-2 (IL-2)[1,2] or γ-interferon[3] (IFN-γ) and to proliferate[1,2,4]. In addition, recent reports suggest that anti-idiotype on the surface of a cell renders that cell sensitive to recognition and lysis by an idiotype-positive CTL clone: B-cell hybridomas producing anti-idiotype serve as targets for idiotype-positive CTL[5,6], and anti-T3 antibodies (T3 is associated with the T-cell receptor) fixed covalently to cells convert them to sensitive CTL targets (H. Spitz, cited in ref. 7).

Recently, we have produced a murine monoclonal antibody specific for an allotypic determinant present on the T-cell receptor of ~25% of peripheral T lymphocytes[8]. The antibody was generated from a C57L/J mouse that had been hyperimmunized with BALB.B peripheral T cells. This monoclonal antibody, F23.1, seems to be very similar in specificity to a rat monoclonal antibody directed against a T-cell receptor isolated from a T hybridoma[9,10]. To determine whether F23.1, the anti-allotypic monoclonal antibody, could focus CTL onto a target cell, we directly coupled the surface of various cell types to the isolated monoclonal antibody using a heterobifunctional crosslinker. OE4 is a CTL clone of C57BL/6 (H-2^b) origin with lytic specificity for H-2^d-bearing cells. The antigen receptor of clone OE4 bears the allotypic determinant recognized by F23.1. Figure 1 shows that OE4 does not kill cells from the lymphoma S.AKR (derived from AKR/J (H-2^k) mice) in a 4-h cytotoxicity assay, but does efficiently lyse P815 (H-2^d) targets. However, S.AKR cells that had been chemically coupled to F23.1 monoclonal antibody were efficiently lysed by OE4. Target cells coupled to the anti-idiotypic monoclonal antibody F9 (which does not recognize OE4), were insensitive to OE4 effector cells, as were S.AKR cells coupled with an anti-H-2^b monoclonal antibody, 20-8-4, which does react with the H-2^b molecules expressed by OE4. Reciprocal results were obtained with the F9 idiotype-positive, allotype-negative CTL clone G4 (BALB.B (H-2^b) anti-H-2^d). In this case, F9-coupled cells were sensitive targets whereas F23.1- and 20-8-4-coupled cells were not killed (data not shown). Thus, we confirmed that monoclonal antibodies directed against determinants on the T-cell antigen receptor can target cells for attack by CTL when coupled to the surface of the cells. Furthermore, an anti-H-2 monoclonal antibody which reacts with the CTL did not trigger lysis.

We next asked whether heteroconjugates of monoclonal antibodies would be able to specifically target cells for destruction by CTL. In the hybrid antibodies that we constructed, one of the monoclonal antibodies in the hybrid is directed at the T-cell receptor (anti-idiotype or anti-allotype) while the other monoclonal antibody is directed at a surface antigen expressed by the targeted tumour cell but not by the CTL. We chose as the targeting monoclonal antibody 19E12, which is specific for the Thy-1.1 alloantigen[11]. The CTL clones G4 and OE4 both express Thy-1.2 but not Thy-1.1 and do not react with this monoclonal antibody. The target cell was S.AKR (H-2^k, Thy-1.1). We

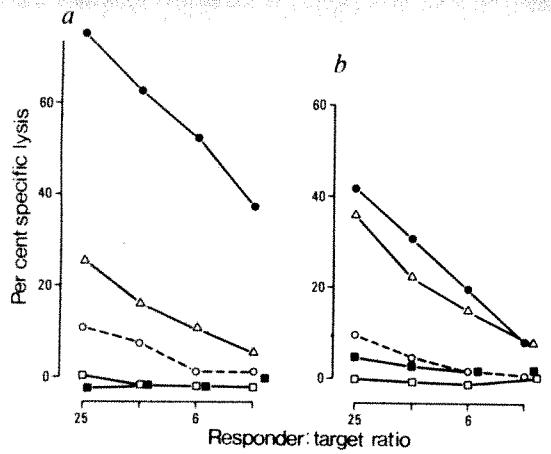


Fig. 3 Cytotoxic effects of Con A-induced (*a*) and F23.1-induced (*b*) spleen cells on various targets. Spleen cells from normal BALB.K (H-2^k) mice were cultured for 4 days at 1×10^6 cells ml^{-1} in RPMI 1640 medium plus 10% fetal calf serum and 10% Con A-induced rat spleen cell supernatant, with either 2.5 $\mu g\ ml^{-1}$ Con A (*a*) or F23.1 monoclonal antibody coupled to Sepharose beads (10^3 beads per ml; *b*). F23.1 cultures also contained 50 mM α-methyl-D-mannoside to block the action of residual Con A present in the rat spleen supernatant. Cells were collected, washed and serial twofold dilutions assayed for lysis of the following ^{51}Cr-labelled target cells: P815 (○), P815 in the presence of 10 $\mu g\ ml^{-1}$ PHA (●), AKR.A1 (□), AKR.A1 cells pre-incubated with 19E12 (anti-Thy-1.1)/F23.1 conjugate (△), and AKR.A1 cells pre-incubated with the 19E12/F9 conjugate (■). AKR.A1 is a T-cell lymphoma derived from an AKR/J mouse which expresses H-2^k and Thy-1.1.

constructed two hybrid monoclonal antibodies using the cross-linker *N*-succinimydyl-3-(1-pyridyldithio) propionate (SPDP). Both hybrid A (19E12/F9) and hybrid B (19E12/F23) worked efficiently and specifically (see Fig. 2). Hybrid A targeted S.AKR cells for lysis by G4 effector cells (Fig. 2*b*) while hybrid B targeted S.AKR cells for lysis by OE4 effector CTL (Fig. 2*d*). Neither hybrid antibody targeted EL4 (H-2^b, Thy-1.2) cells for lysis. Thus, targeting via an antibody combining site works extremely well. Mixtures of unconjugated monoclonal antibodies did not allow target lysis under any circumstances and fractionation of the heteroconjugated monoclonal antibodies on a Sephacryl S-400 column revealed a major peak of specific targeting activity eluting at a relative molecular mass (M_r) of ~300,000, a smaller peak at M_r~500,000 and no targeting activity at lower or higher M_r ranges (data not shown). Other experiments have shown that EL4 cells, which served as the negative control in Fig. 2, can themselves be targeted for lysis by the syngeneic CTL clone OE4 by first infecting them with vesicular stomatitis virus and using an F23/anti-virus G protein monoclonal antibody heteroconjugate.

With regard to potential therapeutic applications of this hybrid antibody targeting technique, it would be beneficial to be able to generate effector T cells carrying a monoclonal antibody-defined receptor determinant from any individual. The anti-allotypic monoclonal F23.1 reacts with ~25% of peripheral T cells (both CTL and non-cytolytic T cells[8,12]), is mitogenic for resting, peripheral T cells from mouse strains which are allotype-positive, and induces effector CTL which bear the allotypic determinant (U.D.S. and M.J.B., unpublished). Figure 3 shows the cytotoxic activity of BALB.K (H-2^k) spleen cells that were induced with either the pan-T-cell mitogen concanavalin A (Con A) or with F23.1 monoclonal antibody coupled to Sepharose beads for 4 days in the presence of exogenous IL-2. Con A induces CTL and T-helper cells regardless of their antigen specificity. The CTL activity can be assayed in an antigen-independent fashion by their ability to lyse any target cell coated with an agglutinant such as Con A or phytohaemagglutinin (PHA)[13]. Figure 3*a* shows that Con A-induced BALB.K cells do not specifically lyse P815 or AKR.A1 (H-2^k, Thy-1.1) targets but do lyse such targets in the presence

Table 1 Monoclonal antibody-induced cytotoxic cells express Thy-1

Target cells	Con A-induced effectors		F23.1-induced effectors	
	C′-treated	Anti-Thy-1+C′	C′-treated	Anti-Thy-1+C′
P815+PHA	76.5	0.4	39.8	−0.6
AKR.A1+19E12/F23	24.2	−0.8	36.7	−0.9

Spleen cells from normal BALB.K mice were cultured for 4 days in the presence of 2.5 $\mu g\ ml^{-1}$ Con A, or F23.1 monoclonal antibody covalently coupled to Sepharose beads plus 10% supernatant of rat spleen cells which had been cultured with Con A for 24 h as a source of IL-2. Cultures not containing Con A also included 50 mM α-methyl-D-mannoside to block the effect of Con A. Cells were collected and treated with complement (C′) alone or with monoclonal anti-Thy-1.2 (ref. 14) plus C′, and surviving cells were assayed for 4 h on ^{51}Cr-labelled target cells. Target cells were P815 in the presence of 10 $\mu g\ ml^{-1}$ PHA or AKR.A1 cells that had been incubated with a hybrid antibody (19E12/F23) for 20 min and washed. AKR.A1 is a T-cell lymphoma from AKR/J mice which expresses H-2^k and Thy-1.1. Spleen cells cultured without a stimulus had <1% of the cytotoxic activity shown here on either target. Assays were performed at a killer/target cell ratio of 25:1 for the C′-treated cells and for the equivalent number of surviving cells from the anti-Thy-1-treated groups. Values are per cent specific lysis of the target cells[3].

of PHA. A fraction of the lytic activity revealed by PHA can also be measured on AKR.A1 cells targeted by the 19E12/F.23 hybrid antibody. In the case of F23.1-induced spleen cells (Fig. 3*b*), PHA plus P815 and the hybrid antibody targeting AKR.A1 cells reveal approximately equal amounts of lytic activity. The levels of lytic activity correlate well with other data showing that the bulk of F23.1-induced T cells express the F23.1 determinant whereas only ~20% of Con A-induced T cells express the F23.1 determinant. As has been shown previously for lectin-induced, lectin-revealed cytotoxicity[13], all of the monoclonal antibody-induced cytotoxicity is sensitive to treatment with anti-Thy-1 antibody plus complement, that is, is due to T cells (Table 1).

Although we do not know whether target cell destruction in this hybrid antibody-directed system proceeds via the same lytic mechanism as in antigen-specific, CTL-mediated killing, we suspect that the mechanism is very similar for the following reasons: first, the T-cell clones that we used are conventional CTL clones (Thy-1^+, Lyt-2^+ cells, whose growth is dependent on a supply of exogenous IL-2 plus periodic antigen stimulation). Second, in the case of Con A-induced effector cells, we have also shown that all of the antibody-directed cytotoxicity is mediated by Lyt-2^+ T cells. Finally, target cell death in this system has the same kinetics and requirement for cell contact as in antigen-specific CTL-mediated lysis.

The results presented here suggest that it is possible to focus a strong T-cell response to a particular target antigen using hybrid antibodies; this could have clinical significance in the rejection of tumour cells or viral infections. One requirement is an antibody against the chosen target, for example, a tumour-associated or viral antigen; the other requirement is an antibody against the T-cell receptor complex. This technique circumvents the failure of the patients' T cells to respond in an effective way to the antigenic challenge (if any) with a specific, histocompatibility locus antigen (HLA)-restricted response. One can envisage inducing the patient's own T cells *in vitro* with an antibody against a receptor determinant, then transferring them back to the patient in the presence of an anti-target/anti-receptor hybrid antibody. We are presently assessing the *in vivo* applications in a murine lymphoma model system, by growing AKR/J (Thy-1.1) T lymphomas in AKR/Cum (Thy-1.2) hosts and using the hosts' own effector T cells and a 19E12/F23 hybrid antibody.

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1. Kappler, J., Kubo, R., Haskins, K., White, J. & Marrack, P. *Cell* **34**, 727–737 (1983).
2. Meuer, S. C. *et al. J. exp. Med.* **158**, 988–993 (1983).
3. Staerz, U. D., Pasternack, M. S., Klein, J. R., Benedetto, J. D. & Bevan, M. J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1799–1803 (1984).
4. Kaye, J., Porcelli, S., Tite, J., Jones, B. & Janeway, C. A. *J. exp Med.* **158**, 836–856 (1983).
5. Ertl, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7479–7483 (1982).
6. Lancki, D. W. & Fitch, F. W. *Fedn Proc.* **43**, 1659 (1984).
7. Martz, E. *Immun. Today* **5**, 254–255 (1984).
8. Staerz, U. D., Rammensee, H.-G., Benedetto, J. D. & Bevan, M. J. *J. Immun.* (in the press).
9. Haskins, K. *et al. J. exp. Med.* **160**, 452–471 (1984).
10. Roehm, N. *et al. Cell* **38**, 577–584 (1984).
11. Houston, L. L., Nowinski, R. C. & Bernstein, I. D. *J. Immun.* **125**, 837–843 (1980).
12. Staerz, U. D. & Bevan, M. J. *ICSU Short Rep.* **2** (in the press).
13. Bevan, M. J. & Cohn, M. *J. Immun.* **114**, 559–565 (1975).
14. Marshak-Rothstein, A. *et al. J. Immun.* **122**, 2491–2497 (1979).
15. Ozato, K. & Sachs, D. *J. Immun.* **126**, 317–321 (1981).
16. Carlsson, J., Drevin, H. & Axen, R. *Biochem. J.* **173**, 723–737 (1978).
17. Jou, Y.-H. & Bankert, R. B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2493–2496 (1981).
18. Chen, S.-S. & Katz, D. H. *J. exp. Med.* **157**, 772–788 (1983).

Gene rearrangement in cells with natural killer activity and expression of the β-chain of the T-cell antigen receptor

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The mammalian host defence system can be divided broadly into adaptive and non-adaptive immunity. Adaptive immunity is acquired and is mediated by B and T lymphocytes. Non-adaptive immunity is mediated in part by a small subclass of heterogeneous peripheral blood mononuclear cells. This population, termed null cells, consists of haematopoietic precursors and cells mediating natural killer (NK) activity and antibody-dependent cellular cytotoxicity (ADCC). NK cells are a class of non-adherent, non-phagocytic, rapidly cytotoxic lymphocytes which can efficiently lyse a wide variety of tumour cells[1], virally infected cells[2] and immature cell types of normal origin[3]. Despite the broad range of targets, only a limited number of specificities are thought to be involved in target-cell recognition[4–6]. Morphologically, NK cells are large granular lymphocytes[7,8], but they have been shown to exhibit cell-surface markers characteristic of both T cells[9–11] and monocytes[12], raising doubt over their lineage. The recent cloning of the β-chain of the T-cell antigen receptor[13,14] has now allowed us to investigate whether some NK cells are T-cell-related. We have examined rearrangement and expression of the β-chain of the T-cell receptor in cloned murine NK cell lines and fresh murine NK cell populations, and our results support the hypothesis that a subpopulation of NK cells is related to T cells and provide a basis for examining whether some NK activity is mediated by a small number of T-cell receptors.

DNA from five independent NK cell lines, HY-1 (ref. 15), HY-3 (ref. 15), NKB61A2 (ref. 16), NKB61B10 (ref. 16) and BALB/c NK (ref. 17), was examined for rearrangement of the genes coding for the β-chain of the T-cell receptor. DNA was digested with six restriction enzymes (*Eco*RI, *Pst*I, *Pvu*II, *Hpa*I, *Hin*dIII or *Xba*I) and examined by Southern gel analysis using a radioactive probe derived from the joining ($J_\beta 1$; *Eco*RI, *Pvu*II and *Hpa*I) or constant ($C_\beta 1$; *Hin*dIII, *Hpa*I, *Pst*I and *Xba*I) regions of the mouse genomic T-cell receptor β-chain genes[18].

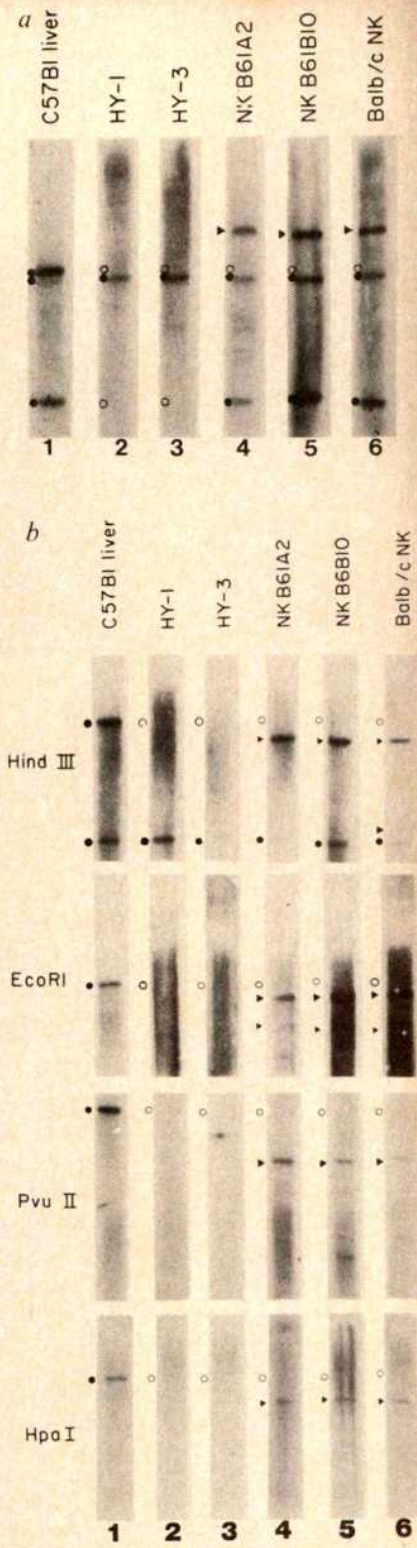


Fig. 1 *a*, Southern blot analysis of DNA from NK cell lines using restriction enzyme *Xba*I. Lane 1, C57BL/6 liver DNA; lane 2, HY-1 DNA; lane 3, HY-3 DNA; lane 4, NKB61A2 DNA; lane 5, NKB61B10 DNA; lane 6, BALB/c NK DNA. *b*, Southern blot analysis of DNA from NK cell lines using T-cell receptor β-chain constant- and joining-region probes. DNA (10 μg) was digested overnight with a 10-fold excess of *Hin*dIII (top panel), *Eco*RI (second panel), *Pvu*II (third panel) or *Hpa*I (bottom panel) (all restriction enzymes from Boehringer Mannheim) and treated as described for *a*, with the exception of the probes. Lane 1, C57BL/6 liver DNA; lane 2, HY-1 DNA; lane 3, HY-3 DNA; lane 4, NKB61A2 DNA; lane 5, NKB61B10 DNA; lane 6, BALB/c NK DNA. Symbols: ●, germline band; ○, bands which have been deleted; ▲, a new, rearranged band.

Methods. *a*, DNA was extracted from the cells using the procedure of Blin and Stafford[24]. DNA (10 μg) was digested overnight with a 10-fold excess of *Xba*I (Boehringer Mannheim) and run on a 6-mm 1% agarose gel (Bio-Rad) at 1.0 V cm^{-1} for 18 h. The DNA was then hybridized with 10^6 c.p.m. ml^{-1} (1 μg of DNA per filter) of nick-translated[25] $C_\beta 1A$ probe of the T-cell antigen receptor β-chain genes for 18 h. $C_\beta 1A$ is the 3.5-kb *Hin*dIII/*Sst*I fragment of *C*1A[18]. This probe contains most of the $C_\beta 1$ coding domains and some 5′-noncoding sequences. Filters were washed and exposed to Kodak XAR-5 films. *b*, *Hin*dIII-digested DNA was hybridized to $C_\beta 1A$ probe (described in *a*) and DNA digested with *Eco*RI, *Pvu*II and *Hpa*I was hybridized to $J_\beta 1$, the insert from the pUCBJ1 subclone of β-chain genes described by Malissen *et al.*[18].

Comparison of the banding pattern of C57BL/6 liver DNA as a control to the NK DNA showed that DNA from all five NK cell lines was rearranged. This result was most evident after digestion by *Xba*I (Fig. 1*a*). There were three germline bands of 5.0, 4.8 and 1.8 kilobases (kb), respectively (Fig. 1*a*, lane 1), and in all cases the 4.8-kb band was retained and the 5.0-kb band deleted. The cell lines HY-1 (Fig. 1*a*, lane 2) and HY-3 (lane 3) had also lost the 1.8-kb band, whereas cell lines

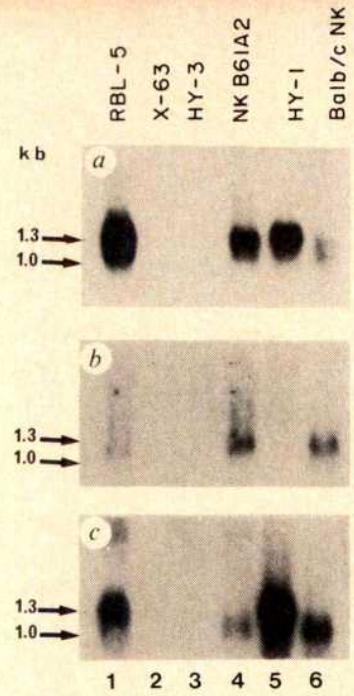


Fig. 2 Northern blot analysis of RNA from NK cell lines using the T-cell receptor β-chain probes. RNA was extracted from cells in the presence of guanidine thiocyanate[26] and 10 μg was treated with glyoxal and electrophoresed through a 1% agarose (Bio-Rad) gel in 10 mM sodium phosphate buffer, *p*H 7.0. Transfer of the RNA to nitrocellulose filters, hybridization and washing were carried out according to the procedure of Thomas[19]. Hybridization was to nick-translated C_β1B (*a*), C_β1U (*b*) or C_β2U (*c*) labelled with ^{32}P-dCTP according to the procedure of Rigby *et al.*[25]. C_β1B is the *Eco*RI/*Eco*RI insert from the pUC8C1B subclone of the cosmid clone 2.3W7 (ref. 18). C_β1U is an ~150-base pair *Eco*RI/*Pst*I fragment consisting of the 3′-untranslated region of C_β1, isolated from RBL-5, a murine cDNA clone[27]. C_β2U is a 2.5-kb *Sst*I/*Sst*I fragment from the cosmid clone 2.3W7[18] which encompasses the 3′-untranslated region of C_β2. Lane 1, RNA from a murine T-cell lymphoma line, RBL-5 (provided by Dr R. Miller); lane 2, RNA from a murine myeloma cell line, X-63 (provided by Dr N. Hozumi); lane 3, HY-3 RNA; lane 4, NKB61A2 RNA; lane 5, HY-1 RNA; lane 6, BALB/c NK RNA.

NKB61A2 (lane 4), NKB61B10 (lane 5) and BALB/c NK (lane 6) had gained a rearranged 6.3-kb band.

We next examined whether either of the two β-chain constant regions was used preferentially in these NK lines. Two probes were used, C_β1A, which hybridizes to both constant regions through the high degree of coding-region homology between them, and J_β1, to confirm that the rearrangement occurs into the first constant region. In the *Hin*dIII digest hybridized to C_β1A probe (Fig. 1*b*, top panel), the 9.8-kb band contained the C_β1 gene whereas the C_β2 gene appeared as a 2.9-kb band (lane 1). In both HY-1 (Fig. 1*b*, lane 2) and HY-3 (lane 3), the C_β1 band had been deleted and no other band appeared, suggesting that the C_β1 gene has been deleted and that C_β2 is the constant region used. In NKB61A2 (Fig. 1*b*, lane 4) and NKB61B10 (lane 5), the C_β1 band was replaced by a 7.8-kb band, and in BALB/c NK (lane 6) the C_β1 band was replaced by 7.8- and 3.5-kb bands. *Eco*RI-digested DNA (Fig. 1*b*, second panel) was hybridized to a J_β1 probe, which does not cross-hybridize to J_β2. The germline band is 9.3 kb (Fig. 1*b*, lane 1). No hybridization was observed to HY-1 (Fig. 1*b*, lane 2) or HY-3 (lane 3) DNA, but in NKB61A2 (lane 4), NKB61B10 (lane 5) and BALB/c NK (lane 6), two new bands of 7.3 and 5.0 kb were found in each lane. DNA digested with *Pvu*II (Fig. 1*b*, panel 3) and *Hpa*I (Fig. 1*b*, bottom panel) was hybridized to J_β1. A single germline band of 11.4 kb (*Hpa*I) or 5.4 kb (*Pvu*II) was replaced by a smaller band of either 9.2 kb (*Hpa*I) or 3.3 kb (*Pvu*II) in NKB61A2, NKB61B10 and BALB/c cell lines.

Table 1 Phenotypic and functional characteristics of spleen cell subpopulations

Sample	Poly-I:C treatment	Selection treatment	NK lytic units per 10^6 cells	% Lyt-1$^+$, 2$^+$ determined by FACS
A	+	None	10	85
B	−	RFC IgG$^+$	210	2
C	+	RFC IgG$^+$	450	1

Enriched and control populations were tested in a 6-h ^{51}Cr-release assay against YAC-1.2 to determine lytic unit values (1 lytic unit is the number of effector cells required for 10% lysis). RFC IgG$^+$, cells forming rosettes with SRBC coated with anti-SRBC were isolated. FACS activity, fluorescence-activated cell sorting using monoclonal anti-Lyt-1, 2 and fluorescein isothiocyanate coupled with F(ab′)$_2$ goat anti-mouse IgG2b.

Our results indicate that all five NK cell lines have undergone rearrangement of the T-cell receptor β-chain genes and that HY-1 and HY-3 have rearrangements into the C_β2 region, whereas NKB61A2, NKB61B10 and BALB/c NK all rearrange into the C_β1 region, with apparently similar rearrangements. We do not know whether these represent similar rearrangements between variable, diversity (*D*) and *J* regions or only between *D* and *J* regions.

We next examined whether RNA homologous to T-cell receptor β-chain genes is expressed in the NK cell lines. Total RNA was isolated from the five NK cell lines, electrophoresed and hybridized, after blotting, to one of three probes: C_β1B, which contains the coding region of C_β1 and some 5′-noncoding sequences; C_β1U, which is a fragment from RBL5 (ref. 18) containing the 3′-untranslated region of C_β1; or C_β2U, containing the 3′-untranslated region of C_β2.

Figure 2*a* shows a Northern blot[19] in which C_β1B was used as a probe; NKB61A2 (lane 4), HY-1 (lane 5), BALB/c NK (lane 6) and NKB61B10 (data not shown) expressed a relatively high level of the β-chain messenger RNA, comparable with that of mouse T-cell lymphoma line RBL-5 (lane 1) used as a control. The B-cell line X-63 (Fig. 2*a*, lane 2), used as a negative control, expressed no C_β1-homologous RNA . HY-3 (Fig. 2*a*, lane 3) expressed a lower level of β-chain mRNA which could be detected only after a longer exposure. Examination of the HY-3 NK cells also revealed a significant decrease in NK killing activity (K.R., unpublished data). Figure 2*b*, *c* shows that BALB/c NK and NKB61A2 express a 1.3-kb C_β1 mRNA and a 1.0-kb C_β2 mRNA, whereas HY-1 and HY-3 seem to express only a 1.3-kb C_β2 mRNA. The NKB61B10 line expressed a 1.3-kb mRNA from the C_β1 region (data not shown). In each case, a full-length 1.3-kb mRNA, presumably containing *V*, (*D*), *J* and *C* sequences[20], was detected. Moreover, the constant regions used in these full-length messages agreed with the data on DNA rearrangement. There was no apparent preference for either of the two constant-region genes in these NK cell lines. The lack of correlation between the presence of a 1.0-kb mRNA and the results of the DNA rearrangement analysis is not surprising, as expression of this short mRNA is known to occur only with *D*–*J* rearrangement[21,22] or no rearrangement at all[20].

To examine whether freshly isolated NK cells also possess these T-cell-like properties, we isolated NK cells from normal mice and from mice stimulated with polyinosinic: polycytidylic acid (poly-I:C). Spleen cells from CBA/J mice were passed over nylon wool columns and NK cells were isolated by recovering cells that form rosettes with sheep red blood cells (SRBC) pretreated with monoclonal IgG2b anti-SRBC antibody. This treatment resulted in a >10-fold increase in the cytolytic activity (lytic units at 10% lysis) against YAC targets and the cells contained <2% Lyt-1$^+$, Lyt-2$^+$ cells as judged by fluorescence-activated cell sorter (FACS) analysis (see Table 1).

Figure 3 shows Northern blot analysis of RNA from these cells. RNA from spleen cells passed over the nylon wool column hybridized to the C_β1B probe for the T-cell receptor (Fig. 3*a*), as the population consists largely (85%) of T cells. The results indicate that enriched NK cells which have not been stimulated

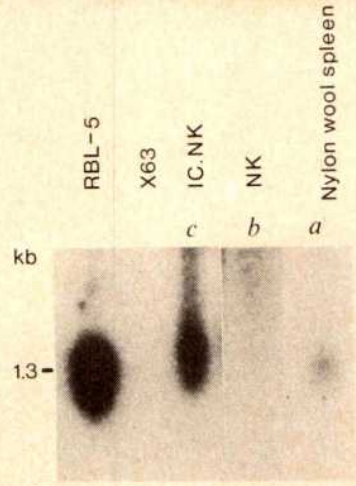


Fig. 3 Northern blot analysis of RNA from NK cells freshly isolated from spleen cells, using T-cell receptor β-chain gene probes. CBA/J mice (4–8 weeks old, $n = 100$) were injected intraperitoneally with 100 μg (0.1 unit) of poly-I:C (Miles Laboratories) per mouse, 24 h before assay (poly-I:C-stimulated mice). A further 100 CBA/J mice were untreated to provide the unstimulated group. Pooled spleen cells were passed over nylon wool columns (*a*), and enriched for NK cells, by direct rosetting with IgG2b-treated SRBC of unboosted (*b*) or poly-I:C-boosted cells (*c*). Nylon-wool-passed cells were rosetted for 1 h at 20 °C with 1% SRBC precoated with 1/50 monoclonal IgG2b anti-SRBC antibody (clone sp2/HL), as described previously[28]. Rosette-forming cells (RFC) were layered onto Ficoll-Hypaque (1.085 g ml^{-1}) and centrifuged for 20 min at 850*g*. Lymphocytes were isolated from the pellet by hypotonic shock. RNA was extracted from 3×10^6 cells and Northern blot analysis was carried out as described in Fig. 2 legend.

with poly-I:C contain very little RNA homologous to the T-cell receptor β-chain (after prolonged exposure) (Fig. 3*b*). However, when poly-I:C-treated spleen cells were enriched and purified for NK cells (Fig. 3*c*), they were found to contain a mRNA of the same size as that of the positive control samples (RBL-5 RNA and spleen T cells; Fig. 3*a*). These preliminary results indicate that freshly isolated NK cells, especially poly-I:C-boosted NK cells, may contain mRNA homologous to the β-chain of the T-cell receptor. It is possible that poly-I:C treatment enriched a population of T-cell-like NK cells, but it is unlikely that the presence of the β-chain mRNA can be attributed to contaminating T cells, as they make up less than 2% (one experiment contained no detectable T cells) of the cell population (Table 1).

We demonstrate here that murine NK cells rearrange and express RNA of the T-cell receptor β-chain genes in five murine NK cell lines and in some cells possessing NK activity which were freshly isolated from murine spleens. These results suggest that at least some NK cells are closely associated with the T-cell lineage. Although HY-1 and HY-3 have been identified as cytotoxic T-cell clones, the others were never so identified. The expression of T-cell receptor-homologous mRNA suggests that the protein product of the rearranged genes may be expressed at the NK cell surface. The finding of a recognition structure, NKTa (relative molecular mass 90,000), on a human NK cell line, which seems to be responsible for its cytolytic activity[23], is consistent with these observations. These findings, however, do not suggest that all NK cell activity can be attributed to T-cell-like NK cells or that the T-cell receptor is necessarily responsible for all NK target cell recognition. The data, however, provide a basis for further examination of whether a limited number of functional rearrangements do indeed occur in these NK cells and also if the rearranged T-cell receptor genes code for a receptor that mediates recognition by NK cells.

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1. Kiessling, R., Klein, E. & Wigzell, H. *Eur. J. Immun.* **5**, 117–121 (1975).
2. Santoli, D., Trinchieri, G. & Leif, F. S. *J. Immun.* **121**, 526–531 (1978).
3. Hansson, M., Kiessling, R., Anderson, B., Karre, K. & Roder, J. *Nature* **278**, 174–176 (1979).
4. Phillips, W. H., Ortaldo, J. R. & Herberman, R. B. *J. Immun.* **125**, 2322–2327 (1980).
5. Allavena, P. & Ortaldo, J. R. *J. Immun.* **132**, 2363–2369 (1984).
6. Roder, J. C., Ahrlund-Richter, L. & Jondal, M. *J. exp. Med.* **150**, 471–481 (1979).
7. Timonen, T., Ortaldo, J. R. & Herberman, R. B. *J. exp. Med.* **153**, 569–581 (1981).
8. Timonen, T., Saksela, E., Ranki, A. & Häyry, P. *Cell. Immun.* **48**, 133–148 (1979).
9. Perussia, B., Fanning, V. & Trinchieri, G. *J. Immun.* **131**, 223–231 (1983).
10. Herberman, R. B., Nunn, M. E. & Holden, H. T. *J. Immun.* **121**, 304–309 (1978).
11. West, W. H., Cannon, G. B., Kaye, H. D., Vonnard, G. D. & Herberman, R. B. *J. Immun.* **118**, 355–361 (1977).
12. Zarling, J. M. & Kung, P. C. *Nature* **288**, 394–396 (1980).
13. Yanagi, Y. *et al. Nature* **308**, 145–149 (1984).
14. Hedrick, S. M., Nielson, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153–158 (1984).
15. Acha-Orbea, H., Groscurth, P., Lang, R., Stiz, L. & Hengartner, H. *J. Immun.* **130**, 2952–2957 (1983).
16. Warner, J. F. & Dennert, G. *Nature* **300**, 31–34 (1982).
17. Dennert, G., Yogeeswaran, G. & Yamagata, S. *J. exp. Med.* **153**, 545–556 (1981).
18. Malissen, M. *et al. Cell* **37**, 1101–1110 (1984).
19. Thomas, P. C. *Proc. natn. Acad. Aci. U.S.A.* **77**, 5201–5205 (1980).
20. Yoshikai, Y. *et al. Nature* **312**, 521–524 (1984).
21. Clark, S. *et al. Nature* **311**, 387–389 (1984).
22. Siu, G. *et al. Nature* **311**, 344–350 (1984).
23. Hercend, T. *et al. J. exp. Med.* **158**, 1547–1560 (1983).
24. Blin, N. & Stafford, D. W. *Nucleic Acids Res.* **3**, 2303–2308 (1976).
25. Rigby, P., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
26. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
27. Caccia, N. *et al. Cell* **37**, 1091–1099 (1984).
28. Beaumont, T. J. *et al. Scand. J. Immun.* **16**, 123–133 (1982).

Rearrangements of the cellular p53 gene in erythroleukaemic cells transformed by Friend virus

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There is now good evidence that the cellular protein, p53, is involved in the transformation process, although its precise role is unknown[1]. It was reported recently that expression of the p53 gene can immortalize cells[2] and that the p53 gene can replace the *myc* oncogene in a *myc–ras* immortalization/transformation assay[3,4]. We have investigated whether p53 is involved in the progression towards the neoplastic state *in vivo* and report here that erythroleukaemic cell lines transformed by different isolates of Friend leukaemia virus show altered expression of the cellular p53 gene. High levels of p53 protein are found in certain lines, but the protein is undetectable in others. This heterogeneity in p53 gene expression is associated with heterogeneity in tumorigenicity. We demonstrate that genomic rearrangements are responsible for p53 gene inactivation in these cell lines and that they occur *in vivo* during the natural progression of Friend virus-induced erythroleukaemia.

Friend leukaemia virus is a complex of two viruses, a replication-defective spleen focus-forming virus (SFFV) and a replication-competent Friend murine leukaemia virus (F-MuLV)[5]. Clonal cell lines transformed by Friend virus were established from methylcellulose colonies derived from individual spleens of Friend virus-infected mice (see Table 1). To test for the presence of p53 protein in these cell lines, cultures were metabolically labelled with ^{35}S-methionine. p53 was immunoprecipitated from cellular extracts, subjected to electrophoresis in SDS-polyacrylamide gels and visualized following autoradiography (Fig. 1). Most (24/31) of the cell lines expressed p53. Of the remaining seven lines, five did not express detectable p53 protein and two expressed a truncated p53-related protein of relative

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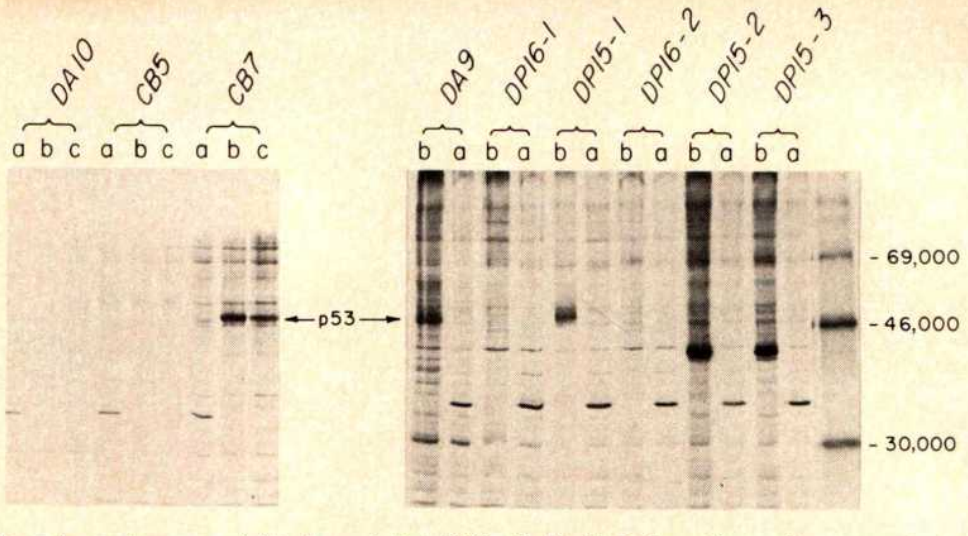


Fig. 1 p53 synthesis in Friend virus-transformed cell lines. Lane *a*, PAb419 (ref. 6) (control monoclonal antibody); lane *b*, PAb421 (ref. 6); lane *c*, RA3-2C2 (ref. 7). Approximately 10^7 cells were labelled for 2 h at 37 °C with 0.2 mCi of ^{35}S-methionine in 0.5 ml α-medium lacking methionine. Cells were then washed with PBS and lysed for 30 min on ice with 0.3 ml lysis buffer (1% Nonidet P-40 (NP40), 150 mM NaCl, 50 mM Tris *p*H 8). Lysates were cleared by centrifugation for 1 min and by the addition of 0.5 ml of a 10% suspension of formalin-treated *Staphylococcus aureus* Cowan 1 cells (Immunoprecipitin, BRL) for 15 min on ice, followed by centrifugation and retention of the supernatant. Lysate volumes corresponding to equal numbers of trichloroacetic-acid-insoluble counts (10^7 c.p.m.) were immunoprecipitated overnight at 10 °C with 40 μl hybridoma tissue culture supernatant in 400 μl NET/gel buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris *p*H 7.4, 0.05% NP40, 0.02% sodium azide, 0.25% gelatine). Immune complexes were collected on 50 μl *S. aureus* cells as above, washed twice in NET/gel buffer and then eluted into 30 μl sample buffer (2% SDS, 10% glycerol, 0.1% bromophenol blue, 25 mM Tris *p*H 6.8) by heating at 70 °C for 10 min. *S. aureus* cells were removed by centrifugation and samples were loaded onto a 12.5% polyacrylamide gel in the presence of SDS. Electrophoresis was at 35 mA until the blue dye front reached the bottom of the gel. Gels were fixed in 7.5% acetic acid/25% methanol for 30 min before drying and exposure to Kodak XAR-5 film.

molecular mass (M_r) ~44,000. These results were confirmed using other monoclonal antibodies recognizing different epitopes on the p53 molecule. The truncated p53-related peptide was recognized by all the monoclonal antibodies tested (data not shown), PAb421 (ref. 6), RA3-2C2 (refs 7, 8), 200.47 (ref. 9) and PAb122 (ref. 10). Note that the cell lines were derived from the spleens of 10 mice and that some lines may, therefore, be sibling clones. The seven lines showing aberrant expression of p53 were derived from four different mice. Thus, the infected spleens from 4 out of 10 mice gave rise to cell lines displaying aberrant p53 expression.

To estimate the steady-state levels of p53 protein in the various cell lines, p53 was immunoprecipitated from increasing amounts of cell extracts and analysed by the Western blotting technique[11]. p53 is detected quantitatively by this procedure (Fig. 2*A*, *B*). A representative autoradiograph shown in Fig. 2*C* confirms the conclusions obtained by metabolic labelling. In cells where p53 protein synthesis was not detected by metabolic labelling, there was at least 100-fold less p53 than in p53-positive Friend cells. p53 was not detected in normal spleen cells (data not shown).

To determine whether the absence of p53 expression, or the synthesis of the antigenically related 44,000-M_r protein, was the result of rearrangement or loss of the cellular p53 gene, genomic DNA from these Friend cell lines was analysed by the Southern blot technique. The probe used, a complementary DNA clone (clone 9 or p27.1a) containing mouse p53-specific sequences[12,13], hybridized to 16.0- and 3.3-kilobase (kb) fragments in *Eco*RI-digested normal mouse DNA or DNA from all the Friend lines expressing p53 (Fig. 3*C*, lanes *b*, *c*, *f*, *j*). The 3.3-kb DNA fragment has previously been shown to contain a p53 pseudogene[14], whereas the 16.0-kb fragment contains the entire

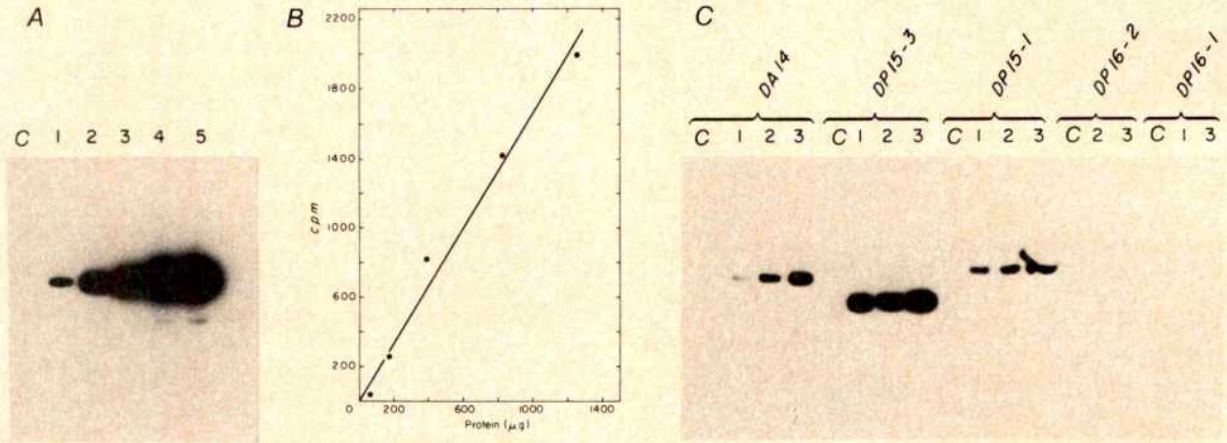


Fig. 2 Quantitative analysis of p53 protein using the Western blotting technique. *A*, Autoradiograph of the blot. Increasing amounts of cell extract prepared from CB7 cells as in Fig. 1 were immunoprecipitated with an excess of PAb421 monoclonal antibodies against p53[6]. Lanes 1–5 contain 100, 200, 400, 800 and 1,200 μg of protein, respectively. Lane *c* contains 400 μg protein immunoprecipitated with a control monoclonal antibody. The number of ^{125}I counts bound to the nitrocellulose paper was measured in a γ-counter and used to generate the dose-response curve shown in *B*. Linear regression analysis was used to fit the best curve to the data. *C*, Increasing amounts of cell extract prepared from several Friend virus-transformed cells were treated as in *A*; lanes 1–3 contain 50, 100 and 200 μg protein, respectively. Lane *c* contains 100 μg protein immunoprecipitated with a control monoclonal antibody. Western blot hybridization was performed essentially as described by Burnette[11] except that immunoprecipitates, instead of crude extracts, were applied to SDS-polyacrylamide gels and then transferred to nitrocellulose. Electroblotting was carried out at 7–8 V cm^{-1} for 6 h. The nitrocellulose was reacted with anti-p53 antibodies (PAb421) and radioiodinated affinity-purified *S. aureus* protein A (Amersham).

Table 1 Tumorigenicity, spleen colony formation and presence of p53 in various Friend virus-transformed cell lines

Cell lines	Presence of p53 (%)	No. of cells injected	Spleen colonies per 10^5 cells	Tumours/ mice injected	Tumour size
DA9	60	10^4	—	6/8	+++
		10^5	26	3/3	+++
		10^6	—	3/3	+++
DA10	<1	10^4	—	8/8	++
		10^5	25	2/3	++
		10^6	—	3/3	++
CB7	100	10^4	—	7/8	+++
		10^5	20	3/3	+++
		10^6	—	3/3	+++
CB5	<1	10^4	—	2/10	++
		10^5	18	—	—
		10^6	—	0/2	—
CB3	<1	10^4	—	1/8	+
		10^5	15	1/3	+
		10^6	—	0/3	—
DP15-1	100	10^4	ND	5/5	+++
DP15-2	<1*	10^4	ND	4/5	+++
DP15-3	<1*	10^4	ND	5/5	+++
DP16-1	<1	10^4	ND	2/5	++
DP16-2	<1		ND	ND	—

The cell lines were established from methylcellulose colonies derived from individual spleens of Friend virus-infected mice as described previously[19]. Cells were maintained in α-medium supplemented with 10% fetal calf serum. Virus stocks of the polycythaemia- and anaemia-inducing strains of Friend virus complex (FV-P and FV-A, respectively) were obtained as follows. NIH 3T3 fibroblasts non-productively infected with the anaemia-[23] or polycythaemia[24]-inducing strains of Friend spleen-focus-forming virus ($SFFV_A$ and $SFFV_P$) were superinfected with cloned Friend murine leukaemia virus (F-MuLV). F-MuLV was obtained from the tissue culture supernatant of NIH 3T3 cells that had been transfected with F-MuLV DNA clone 57 (ref. 25). Cell lines designated DA and DP were derived from FV-A- or FV-P-infected mice, respectively. The cell lines CB3, CB5 and CB7 were derived from newborn BALB/c mice infected with F-MuLV[26]. All other cell lines were derived from DBA/2J mice infected as adults with either FV-A or FV-P. Cell lines with the same number, for example, DP15-1, -2, -3, were derived from different methylcellulose colonies from the same mouse spleen. Cell lines CB3 and CB5 were derived from the same mouse. The presence of p53 protein was determined by the Western blotting technique (see Fig. 2 legend). The amounts are relative to cell line CB7. To count spleen colonies, irradiated (850–900 rad) mice were injected with 10^5 cells in phosphate-buffered saline (PBS). Spleens were removed 9 days later, fixed in Bouin's solution and the colonies counted. The number shown is the average of two spleens. ND, not determined. +++ Refers to large tumours which developed at the site of subcutaneous injection within 2–4 weeks; ++, smaller tumours which developed within 3–6 weeks; +, small tumours which developed 2–3 months after injection.

* These cells contained no detectable p53 protein but did contain high levels of an antigenically related protein of 44,000 M_r that was recognized by monoclonal antibodies to p53 (see Figs 1, 2).

functional p53 gene[15]. Similarly, *Bam*HI or *Hin*dIII digestion of either normal mouse DNA or DNA from p53-producing Friend cell lines resulted in two DNA fragments of 9.0 and 6.0 kb for *Bam*HI and 7.6 and 2.0 kb for *Hin*dIII which hybridized to the p53 probe (Fig. 3*A*, lanes *b*, *c*, *f*, *k*; *B*, lanes *b*, *d*). Thus, at this level of resolution there are no detectable rearrangements of the p53 gene in p53-producing Friend cell lines.

We next examined the seven cell lines that failed to express p53 protein. Digestion of cellular DNA of clone DA10 with the enzymes *Eco*RI, *Bam*HI or *Hin*dIII revealed no rearrangement of the p53 gene (Fig. 3*A*–*C*, lanes *a*). However, p53 mRNA was not detected by Northern gel analysis[16] (data not shown). Thus, the absence of p53 protein can be explained by a transcriptional block in the expression of the p53 gene. In contrast, the other cell lines contained p53 DNA fragments of different sizes. Cell

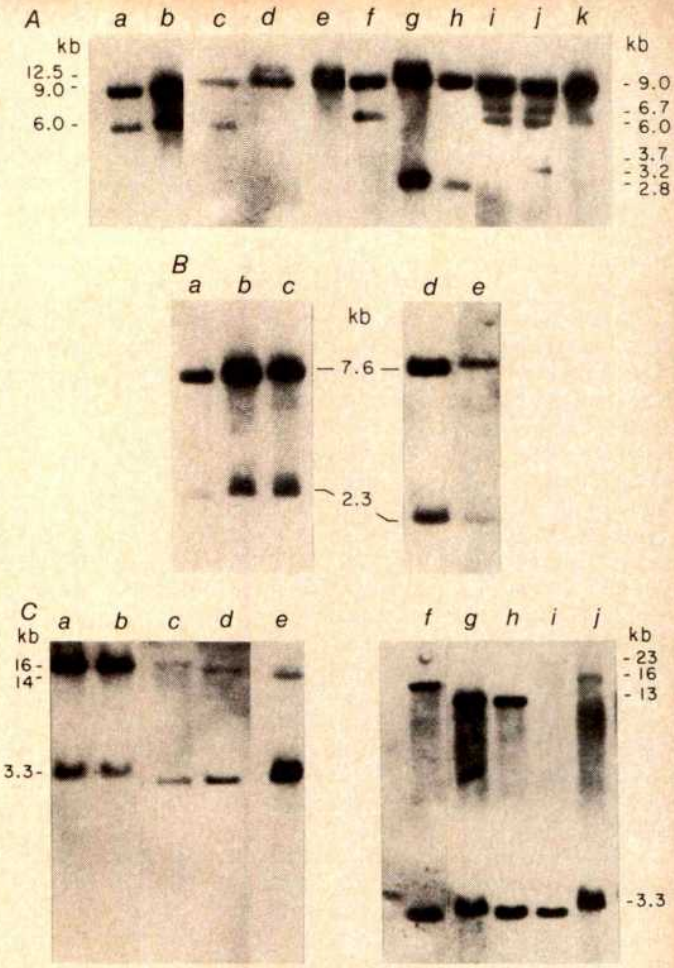


Fig. 3 Genomic DNA blots of Friend virus-transformed cell lines. p53-specific sequences were detected using a p53 cDNA plasmid clone as probe[12,13]. *A*, Genomic DNA from the following cell lines was digested with *Bam*HI: *a*, DA10; *b*, DA9; *c*, CB7; *d*, CB5; *e*, CB3; *f*, DP15-1; *g*, DP15-2; *h*, DP15-3; *i*, DP16-1; *j*, DP16-2; *k*, DP16-3; DP16-3 is a p53-producing cell line. *B*, Genomic DNA from the following cell lines was digested with *Hin*dIII: *a*, DA10; *b*, DA9; *c*, CB3; *d*, CB7; *e*, CB5. *C*, Genomic DNA from the following cell lines was digested with *Eco*RI: *a*, DA10; *b*, DA9; *c*, CB7; *d*, CB5; *e*, CB3; *f*, DP15-1; *g*, DP15-2; *h*, DP15-3; *i*, DP16-2; *j*, DP16-3.

Methods. DNA was isolated on guanidinium isothiocyanate CsCl gradients[27]. CsCl (1 g ml^{-1}) was added to the cell lysate in guanidinium isothiocyanate. The lysate was then loaded on a 5.7 M CsCl cushion and spun at 25,000 r.p.m. for 24 h in a SW27 rotor. The viscous DNA above the cushion was dialysed against TE (10 mM Tris *p*H 8.0, 1 mM EDTA), then extracted with phenol/chloroform. The DNA was precipitated with ethanol, spooled on a glass rod and resuspended in TE and dialysed against TE. DNA was digested and electrophoretically separated in agarose gels as described previously[28]. The DNA in the gels was acid depurinated by the technique of Wahl *et al.*[29], before transfer to zeta probe filters (AMF Corporation) as described by Southern[30]. The filters were hybridized to 1×10^6 c.p.m. ml^{-1} of nick-translated[31] p53 cDNA plasmid[12,13] in 50% formamide and 10% dextran sulphate for 16 h at 42 °C. The final wash was at 50 °C in 0.1% sodium lauryl sulphonate and 0.1 × SSC (1 × SSC = 0.15 M NaCl plus 0.015 M sodium citrate).

lines CB5, CB3, DP15-2 and DP15-3 have lost both alleles corresponding to the normal 6.0-kb *Bam*HI fragment (Fig. 3*A*, lanes *d*, *e*, *g*, *h*); instead, p53 fragments of ~12.5 kb were observed after *Bam*HI digestion of CB3 or CB5 DNA. Similarly, digestion of DP15-2 and DP15-3 DNA with *Bam*HI produced a p53 DNA fragment of 2.8 kb (Fig. 3*A*, lanes *g*, *h*). Digestion of DNA from these same cell lines with *Eco*RI confirmed both the presence of a rearrangement in the functional p53 gene and the loss of the normal homologous allele (Fig. 3*C*).

Cell clone DP16-2 also has a rearranged p53 gene, as demonstrated by the loss of the 16-kb *Eco*RI fragment and the appearance of a novel 23-kb DNA fragment (Fig. 3*C*, lane *i*). Digestion of DP16-1 and DP16-2 DNA with *Bam*HI resulted in a complex pattern of p53 DNA fragments (Fig. 3*A*, lanes *i*, *j*), suggesting that independent and different genomic rearrangements occurred at each homologous allele to inactivate the p53 gene. Structural alterations in the p53 pseudogene were not observed. We conclude that rearrangement of the p53 gene is involved in gene inactivation and in the production of the truncated 44,000-M_r protein.

Because several Friend lines examined here were established from methylcellulose colonies derived from the same mouse, we were able to investigate whether independent *in vitro* colonies were clonally related by examining their unique p53 rearrangements. For example, cell lines DP15-2 and DP15-3 are derived from the same DBA mouse infected with FV-P. These cell lines both express a 44,000-M_r protein antigenically related to p53 and have an identical rearrangement of their p53 gene. Similarly, cell lines DP16-1 and DP16-2 share identical rearrangement of their p53 gene (but different from DP15-2 and DP15-3). Cell clones CB3 and CB5 are also derived from the same BALB/c mouse and share identical rearrangement of their p53 gene and common F-MuLV integration sites (data not shown). The presence of unique p53 rearrangements in more than one cell line derived from the same mouse suggests that the genomic p53 rearrangements described here occurred *in vivo* and were not an artefact of cell culture. Thus, we conclude that the genomic rearrangements must have conferred selective advantage on these cells during the natural progression of Friend disease *in vivo*.

It has previously been reported that expression of p53 protein is associated with tumorigenicity in Abelson virus-transformed lymphoid cell lines[17,18]. The availability of Friend cell lines differing in p53 expression allowed us to investigate whether this heterogeneity was reflected in tumorigenic phenotype. Table 1 compares the ability of the various Friend virus-transformed cell lines to form subcutaneous tumours in syngeneic adult mice. BALB/c mice injected subcutaneously with the p53 producer cell line CB7 had large tumours, whereas no tumours were detected after injection with either of the p53 non-producer cell clones CB3 or CB5. After longer latency periods (1.5–3 months), occasional tumours were observed in these mice. None of the tumours formed by CB3, CB5 or CB7 regressed.

The efficiency of tumour formation by the p53 non-producer cell line DA10 was similar to that observed with the p53 producer cell DA9, although there was a 1–2-week delay in the time when tumours were first detected with DA10. Also, DA10 tumours were much smaller than tumours formed by DA9.

To investigate whether the differences in tumorigenicity between the p53 producer and non-producer Friend cell lines reflected differences in the immunogenicity or *in vivo* growth requirements of these cells, rather than a direct effect of p53 on tumour phenotype, we utilized the fact that Friend virus-transformed cell lines are able to form spleen colonies in irradiated syngeneic hosts[19,20]. No differences were observed in either the spleen colony-forming efficiency or spleen colony size between p53 producer and p53 non-producer cell lines (Table 1).

To determine whether the tumours that formed from p53 non-producer cell lines were variants or revertants that had now acquired the ability to express p53 protein, we analysed the subcutaneous tumours formed by p53-negative and -positive cell lines by the Western blotting technique. No p53 protein was detected in tumours formed by cell lines DA10, CB3, CB5 and DP16-1, whereas p53 was detected in tumours from cell lines CB7 and DA9 (data not shown). Thus, expression of p53 protein is not essential for tumour formation *in vivo*; rather, the presence of this protein in Friend virus-transformed cells seems to enhance their tumour phenotype.

Because high levels of p53 protein have been reported previously in four different Friend cell lines[21], we were surprised to find no p53 expression in cell lines derived from the infected spleens of three mice. The cell lines used in the previous study were all derived after subcutaneous passage of transplantable tumours whereas the Friend virus-transformed cell lines examined in our study were from colonies obtained by plating spleen cells from mice infected with Friend virus in methylcellulose. Thus, unlike the earlier study[21], there was no direct selection for tumorigenicity placed on the cell lines used here.

The high frequency of p53 gene inactivation and rearrangement observed in Friend erythroleukaemic cells is intriguing and unexpected. Moreover, it suggests that inactivation of the p53 gene is not a random event, but is closely associated with the induction of erythroleukaemia by Friend virus complex or its helper virus. Note, however, that in the p53-producing cell lines examined here, the amount of p53 protein is at least 100-fold higher than in normal spleen cells. Thus, transformation of erythroid cells by Friend virus is accompanied by major changes in the regulation of p53 protein levels—p53 protein levels may rise ~100-fold or expression of the p53 gene may be completely shut-off through gene inactivation. In the latter case, neither cell division nor immortalization functions seem to be affected.

We cannot explain why many tumour cells express high levels of p53 protein whereas some of the Friend cell lines described here, as well as an Abelson virus-transformed cell line described previously[22], have genomic rearrangements leading to p53 gene inactivation. Resolution of this paradox may provide some insights into the roles of p53 in normal and malignant cells.

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1. Crawford, L. *Int. Rev. exp. Path.* **25**, 1-50 (1983).
2. Jenkins, J. R., Rudge, K. & Currie, G. A. *Nature* **312**, 651-654 (1984).
3. Parada, L. F., Land, H., Weinberg, R. A., Wolf, D. & Rotter, V. *Nature* **312**, 649-651 (1984).
4. Eliyahn, D., Raz, A., Gruss, P., Givol, D. & Oren, M. *Nature* **312**, 646-649 (1984).
5. Teich, N. *et al.* in *RNA Tumor Viruses* (eds Weiss, R., Teich, N., Varmus, H. & Coffin, J.) 857-880 (Cold Spring Harbor Laboratory, New York, 1982).
6. Harlow, E., Crawford, L. V., Pim, D. C. & Williamson, N. M. *J. Virol.* **39**, 861-869 (1981).
7. Coffman, R. L. & Weissman, I. L. *J. exp. Med.* **153**, 269-279 (1981).
8. Rotter, V., Boss, M. A. & Balimore, D. *J. Virol.* **38**, 336-346 (1981).
9. Dippold, W. G., Jay, G., Deleo, A. B., Khoury, G. & Old, L. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1695-1699 (1981).
10. Gurney, E. G., Harrison, R. O. & Fenno, J. *J. Virol.* **34**, 752-763 (1980).
11. Burnette, W. N. *Analyt. Biochem.* **112**, 195-203 (1981).
12. Benchimol, S. *et al.* in *Cancer Cells* Vol. 2 (eds Levine, A., Topp, W., Vande Woude, G. & Watson, J. D.) 383-391 (Cold Spring Harbor Laboratory, New York, 1984).
13. Jenkins, J. R., Rudge, K., Redmond, S. & Wade-Evans, A. *Nucleic Acids Res.* **12**, 5609-5626 (1984).
14. Zakut-Houri, R. *et al. Nature* **306**, 594-597 (1983).
15. Bienz, B., Zakut-Houri, R., Givol, D. & Oren, M. *EMBO J.* **3**, 2179-2183 (1984).
16. Thomas, P. S. *Meth. Enzym.* **100**, 255-266 (1983).
17. Rotter, V., Abutbul, H. & Wolf, D. *Int. J. Cancer* **31**, 315-320 (1983).
18. Wolf, D., Harris, N. & Rotter, V. *Cell* **38**, 119-126 (1984).
19. Mager, D. L., Mak, T. W. & Bernstein, A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1703-1707 (1981).
20. Mager, D. L., Mak, T. W. & Bernstein, A. *Nature* **288**, 592-594 (1980).
21. Ruscetti, S. K. & Scolnick, E. M. *J. Virol.* **46**, 1022-1026 (1983).
22. Wolf, D. & Rotter, V. *Molec. cell. Biol.* **4**, 1402-1410 (1984).
23. MacDonald, M. E. *et al. J. exp. Med.* **151**, 1477-1492 (1980).
24. Bernstein, A., Mak, T. W. & Stephenson, J. R. *Cell* **12**, 287-294 (1977).
25. Oliff, A. *et al. J. Virol.* **33**, 475-486 (1980).
26. Shibuya, T. & Mak, T. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3721-3725 (1983).
27. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294-5299 (1979).
28. Mowat, M. & Bernstein, A. *J. Virol.* **47**, 471-477 (1983).
29. Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683-3687 (1979).
30. Southern, E. *J. molec. Biol.* **98**, 503-517 (1975).
31. Rigby, P. W. J., Diekmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).

α-Interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences

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Complementary DNAs corresponding to the interferon (IFN)-induced messenger RNAs for histocompatibility locus antigens (HLA), metallothionein-II (MT2), 2′,5′-oligoadenylate synthetase and about eight other proteins of unknown sequence have been isolated recently[1–5], and by interferon regulation of transcription has been demonstrated for several ofthe eight mRNAs[3,4], with a significant increase apparent in as little as 5 min[3]. We now show that IFN-α treatment results in a three- to fivefold increase in the transcription of *MT2* and *HLA* class I genes in human T98G neuroblastoma cells. Furthermore, comparison of regions upstream of the *MT2A* gene, two *HLA* genes and one *HLA* class II gene reveals a homologous sequence of ~30 base pairs (bp) which may be involved in regulating transcription of interferon-induced genes. Transcription of the mRNA for human *MT2A* is induced by glucocorticoids or metal ions[6,7] and the regulatory elements have been mapped by promoter-fusion experiments[8]. We now show that the rate of transcription of *MT2A* is the same on treatment with interferon or dexamethasone, but that the mRNA accumulates much faster with dexamethasone, indicating that post-transcriptional events are important in the latter case.

We determined relative rates of transcription in isolated nuclei by hybridizing ^{32}P-labelled RNA, synthesized in run-off transcription reactions, to cDNA probes bound to nitrocellulose filters. The assay measures the relative number of transcriptional complexes initiated on genes corresponding to the complementary DNA probes (see ref. 3 for experimental details and discussion of this method). To measure transcription from *HLA* class I genes, we used plasmid pABC001 (ref. 9), containing *HLA-B7* cDNA that hybridizes to *HLA-A*, *HLA-B* and *HLA-C* mRNAs. The *1-8* and *MT2* cDNAs used were isolated by Friedman *et al.*[3]. A full-length human β-actin cDNA[10] was used as a control in all experiments.

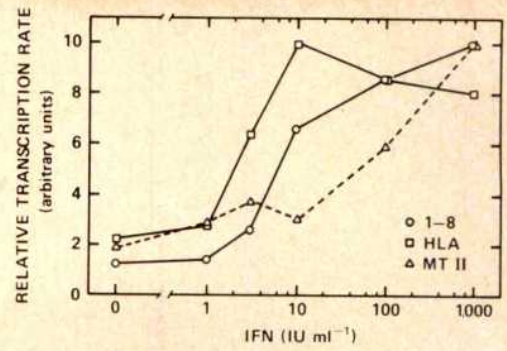


Fig. 1 Dependence of transcription on IFN-α concentration. T98G cells were treated with IFN-α for 2 h and relative transcription rates were measured in the nuclear run-off assay. Duplicate assays agreed to ±20%. To facilitate comparisons, the highest point in each assay was assigned a relative rate of 10. T98G human neuroblastoma cells were grown as described previously[3]. Wellferon, a highly purified mixture of IFN-α, specific activity $1–2\times10^8$ IU ml^{-1} (ref. 23), was used in all experiments. Transcription assays were modified slightly from those used by Friedman *et al.*[3] in that hybridizations were carried out in a volume of 300 μl, containing 1.5×10^7 c.p.m. of ^{32}P-labelled RNA.

Interferon-induced transcription of *1-8*, *HLA* and *MT2* in a dose-dependent manner (Fig. 1), while transcription of the β-actin gene was not affected by any IFN-α concentration tested. Half-maximal induction of *MT2* transcription required 30 times more IFN-α than similar induction of *HLA* genes. We have shown previously that the accumulation of six mRNAs is a function of interferon concentration over a wide range and that the concentration of IFN-α for half-maximal induction is different for different mRNAs. The accumulation of *MT2* mRNA requires a higher concentration of IFN-α than any of the others[3]. We conclude that dose-dependent changes in transcription rate account, at least in part, for the dependence of mRNA accumulation on interferon concentration.

When cells were treated for 13 h with 1,000 IU ml^{-1} IFN-α, the transcription rates of *1-8*, *MT2* and *HLA* genes were about half those observed after 2 h of treatment (Fig. 2*a*). For the *1-8* gene, the extent of this decrease was less than we reported previously[3] at a lower IFN-α concentration (100 IU ml^{-1}), suggesting that the degree of down-regulation of *1-8* transcription may depend on the concentration of IFN-α. We used 1,000 rather than 100 IU ml^{-1} of IFN-α in the current experiments to obtain maximum induction of *MT2*.

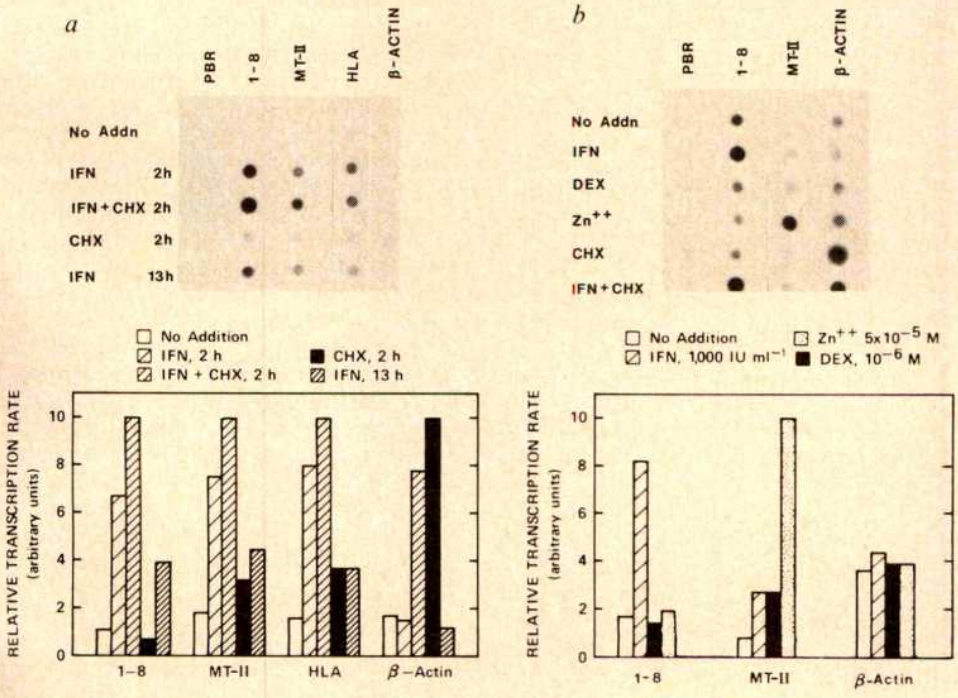


Fig. 2 *a*, Effect of inhibiting protein synthesis or prolonging treatment with IFN-α on interferon-induced transcription. The results of duplicate experiments were averaged. The values agreed to ±15% except for *HLA* genes in cycloheximide (CHX)-treated cells, where the duplicates agreed to ±50%. *1-8* and β-actin served as positive and negative controls, respectively. The concentration of IFN-α was 1,000 IU ml^{-1}. Cycloheximide was used at 32 μg ml^{-1}, a concentration that inhibits protein synthesis in T98G cells by >95%[3]. *b*, Effects of IFN-α (1,000 IU ml^{-1}), dexamethasone (DEX, 1 μM) and Zn^{2+} (50 μM) on transcription. Treatment was for 2 h. The results of duplicate experiments, which agreed to ±15%, were averaged. The highest point for β-actin was assigned a relative value of 5.

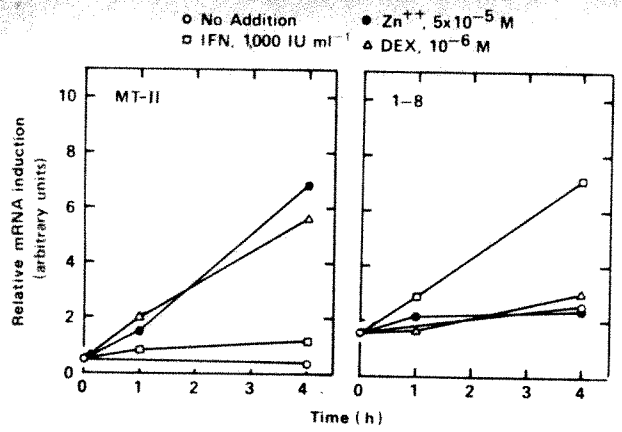


Fig. 3 Effects of IFN-α, dexamethasone and Zn^{2+} on accumulation of *MT2* mRNAs. Levels of mRNA were measured by the slot hybridization technique described previously[3]. One experiment is shown, but similar results were obtained in two additional independent experiments. The increase in *MT2* mRNA after 4 h was 3–5-fold for IFN-α, consistent with the results reported previously[3] and 15–20-fold for Zn^{2+} or dexamethasone. Rabbit β-globin mRNA was added to the lysis buffer to allow correction for differences in RNA recovery to be made. Levels of endogenous β-actin mRNA were also measured. The β-actin and β-globin mRNA levels did not vary significantly with any of the treatments used.

Pretreatment with cycloheximide did not block interferon-induced transcription of the *MT2* or *HLA* genes (Fig. 2*a*), in agreement with our previous result[3] for *1-8*. Cycloheximide alone had little effect on the transcription of these genes, but greatly increased transcription of the β-actin gene, as reported previously[3,11].

The increased transcription of *MT2* after treatment with IFN-α (Fig. 2*a*) shows that control of the *MT2* promoter is more complex than believed previously[8]. To define regulation of *MT2* expression more precisely, we measured mRNA levels and transcription rates following exposure of the cells to IFN-α, dexamethasone no Zn^{2+}; we found that either IFN-α or dexamethasone induced a 3–4-fold increase in the transcription rate of *MT2* (Fig. 2*a*,*b*) while treatment with 50 μM Zn^{2+} produced a 13-fold increase (Fig. 2*b*).

The rate of *MT2* mRNA accumulation was linear for 4 h after treatment with IFN-α, dexamethasone or Zn^{2+} (Fig. 3). We have shown previously[3] that the induction of *MT2* mRNA by interferon is maximal after 12 h. In control experiments, neither dexamethasone nor Zn^{2+} stimulated accumulation of *1-8* mRNA (Fig. 3). Although IFN-α or dexamethasone produced similar increases in *MT2* transcription (Fig. 2*b*), the accumulation of *MT2* mRNA was much greater with dexamethasone (Fig. 3). Treatment with Zn^{2+} increased gene transcription much more than did dexamethasone treatment (Fig. 2*b*), but the rates at which the mRNAs accumulated were very similar (Fig. 3). We conclude that post-transcriptional stabilization and increased transcription both contribute significantly to accumulation of *MT2* mRNA in dexamethasone-treated T98G cells. This effect is selective, because treatment with dexamethasone does not lead to an increase in *1-8* or β-actin mRNA.

Yoshie *et al.*[12] transfected Ltk$^-$ cells with fragments of an *HLA* gene lacking not only the 5′-flanking sequences but also all of the first and part of the second exon. Nevertheless, RNAs corresponding to the fragments were induced on treatment with 1,000 IU ml^{-1} of IFN-α. As shown in Figs 1 and 2, IFN-α induces transcription of class I *HLA* genes, so it is possible that signals for interferon-induced transcription are preserved in the gene fragments of Yoshie *et al.*[12]. However, we have shown previously that post-transcriptional events can contribute to accumulation of the interferon-induced *1-8* mRNA[3]. Therefore, it seems more likely that the results of Yoshie *et al.*[12] are explained by interferon-induced post-transcriptional changes and that the transcription rates of the deleted, transfected *HLA* gene fragments do not change when interferon is added.

HLA-DR	GTGTTGA**ACCTC**-A**G**AGTTTCTC**C**TCT-CAT
HLA-A3	ATTCCCC**A**ACTCC**GCAGTTTCT**TTTCT-CTC
HLA*	ATTCGCC**ACCTCCGCAGTTTCTC**TTCTTCTC
MT-II	TC**TCTGGACCT**--**GCAGTTTCTCCTCT**-CTA
Consensus	TTCN$^{G}_{C}$N**ACCTCNGCAGTTTCTC**$^{C}_{T}$**TCT-CT**

Fig. 4 Homologous 5′ sequences flanking the *MT2* and *HLA* genes. Bold face letters indicate nucleotides conserved in at least three of the four examples. N, any nucleotide. The sequences are from Malissen *et al.*[16], Karin and Richards[24] (with one modification, provided by Dr Michael Karin, personal communication), Das *et al.*[17] and Strachan *et al.*[15] Access to the QUEST, SEQ and IFIND programs was generously provided by IntelliGenetics, a division of IntelliCorp. QUEST was used to retrieve sequences from the NIH and EMBL databases. SEQ was used to find initial intra- and intersequence homologies, for probability calculations and for symmetry searches. The default SEQ parameters were used except for Loopout, which was set to 1. IFIND, based on the algorithm of Wilbur and Lipman[25], was used to make the final alignments, to arrive at a consensus sequence, and to search the NIH and EMBL databases for homologies to the consensus sequence.

Our conclusion that post-transcriptional events are significantly involved in the accumulation of *MT2* mRNA after dexamethasone treatment is similar to that of Vannice *et al.*[13], who showed that dexamethasone produces a marked accumulation of the mRNA for α_1-acid glycoprotein, but no increase in transcription. We suggest a refinement of the idea of Richards *et al.*[14] that differences in sequences 5′ to *MT2* genes account for their differential regulation. Sequences responsible for post-transcriptional regulation are probably also involved.

Because IFN-α regulates transcription of the *MT2* and *HLA* genes, we compared the sequences upstream from *MT2* with sequences upstream from the three *HLA* genes for which such information is available—a class I *HLA-A3* gene[15], an unidentified class I gene[16] and a class II *HLA-DR*α gene[17]. Class I genes are efficiently induced by both IFN-α and IFN-γ, whereas the *HLA-DR*α gene is efficiently induced by IFN-γ and poorly induced by IFN-α[5]. The cap sites for *HLA-A3* and the gene of Malissen *et al.*[16] have not been mapped. By analogy to the *HLA-DR* sequence, we tentatively assign the CAT (CCAAT) and TATA (TCTAAA) boxes to positions 162 and 188 in the *HLA-A3* sequence and to positions 156 and 182 in the sequence of Malissen *et al.*[16].

The two *HLA* class I sequences share considerable homology upstream of their 5′ start sites. There is also homology between a region about 760 bp 5′ to the start site of the *MT2A* gene and a region about 250 bp 5′ to the putative start site in the sequence of Malissen *et al.*[16] (Fig. 4). As calculated with the SEQ program, the probability of finding such a homology by chance is 0.018, taking the base compositions into account. A similar region of homology was also found for *HLA-A3* and *HLA-DR*α (Fig. 4). The only significant homology between sequences 5′ to the two *HLA* class I genes and the class II *HLA-DR*α gene was found in the region also homologous to *MT2*. Alignment reveals that AGTTTCTC$^{C}_{T}$TCTC, the core of the consensus sequence, is nearly invariant and that the first seven bases of this core are absolutely invariant. The consensus sequence lies 142 or 147 bp upstream of the TATA box of the class I gene of Malissen *et al.*[16] or the *HLA-A3* gene, respectively. It is 600 or 567 bp upstream of the *MT2* or *HLA-DR*α TATA box, respectively, and there is no dyad symmetry near any of the consensus sequences. We also compared the consensus sequence with all human and murine sequences in the databases of the National Institutes of Health (NIH) and the European Molecular Biology Laboratory (EMBL). Only the *HLA* and *MT* sequences used to derive the consensus showed significant homology to it, indicating that the consensus is not merely a sequence repeated frequently in genomic DNA.

The consensus sequence extends over 28 bp and is considerably longer than the sequences proposed to be involved in glucocorticoid-mediated induction of mouse mammary tumour

virus (TGGTACAAAATGTTCT) or *MT2* transcription[8,14], or in metal ion induction[8] of *MT2* ($^{TG}_{GT}$CGCCCGGC$^{T}_{C}$C). It is also more extensive than the consensus sequence for transcriptional regulation of heat shock genes (GTNGAANNTTCNAG) which has been shown to function in mammalian cells, *Xenopus* oocytes and *Drosophila*[18-20]. General regulation of yeast amino-acid biosynthesis also depends on a short regulatory sequence (A^{A}_{T}GTGACTC)[21,22]. The sequences involved in regulation of transcription by glucocorticoids, metal ions or heat shock are conserved among different species. We have found no homology between the consensus sequence shown in Fig. 4 and sequences 5′ of mouse *H-2* genes, but the mouse sequences available in the NIH and EMBL databases do not extend far beyond the 5′ start sites of the mRNAs.

Experiments are now under way to test directly the role of the consensus sequence in transcriptional regulation by interferon. If this sequence is involved, it may be long enough to be used to isolate genes directly from phage or cosmid libraries by hybridization, and would help in locating their regulatory regions. Both consensus and naturally occurring sequences might then be used to isolate the proteins responsible for interferon transcriptional regulation.

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1. Chebath, J., Merlin, G., Metz, R., Benech, P. & Revel, M. *Nucleic Acids Res.* **11**, 1213-1226 (1983).
2. Merlin, G., Chebath, J., Benech, P., Metz, R. & Revel, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4904-4908 (1983).
3. Friedman, R. L., Manly, S. P., McMahon, M., Kerr, I. M. & Stark, G. R. *Cell* **38**, 745-755 (1984).
4. Larner, A. C. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 6733-6737 (1984).
5. Rosa, F. *et al. EMBO J.* **2**, 1583-1589 (1983).
6. Karin, M., Andersen, R. D., Slater, E., Smith, K. & Herschman, H. R. *Nature* **286**, 295-297 (1980).
7. Karin, M., Andersen, R. D. & Herschman, H. R. *Eur. J. Biochem.* **118**, 527-531 (1981).
8. Karin, M. *et al. Nature* **308**, 513-519 (1984).
9. Sood, A. K., Pereira, D. & Weissman, S. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 616-620 (1981).
10. Gunning, P. *et al. Molec. cell. Biol.* **3**, 787-795 (1983).
11. Ringold, G. M., Dieckmann, B., Vannice, J. L., Trahey, M. & McCormick, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3964-3968 (1984).
12. Yoshie, O. *et al. Proc. natn. Acid. Sci. U.S.A.* **81**, 649-653 (1984).
13. Vannice, J. L., Taylor, J. M. & Ringold, G. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4241-4245 (1984).
14. Richards, R. I., Heguy, A. & Karin, M. *Cell* **37**, 263-272 (1984).
15. Strachan, T., Sodoyer, R., Damotte, M. & Jordan, B. R. *EMBO J.* **3**, 887-894 (1984).
16. Malissen, M., Malissen, B. & Jordan, B. R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 893-897 (1982).
17. Das, H. K., Lawrence, S. K. & Weissman, S. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3543-3547 (1983).
18. Bienz, M. & Pelham, H. R. B. *EMBO J.* **1**, 1583-1588 (1982).
19. Pelham, H. R. B. *Cell* **30**, 517-528 (1982).
20. Pelham, H. R. B. & Bienz, M. *EMBO J.* **1**, 1473-1477 (1982).
21. Donahue, T. F., Daves, R. S., Lucchini, G. & Fink, G. R. *Cell* **32**, 89-98 (1983).
22. Lucchini, G., Hinnebusch, A. G., Chen, C. & Fink, G. R. *Molec. cell. Biol.* **4**, 1326-1333 (1984).
23. Allen, G., Fantes, K. H., Burke, D. C. & Morser, J. *J. gen. Virol.* **63**, 207-212 (1982).
24. Karin, M. & Richards, R. I. *Nature* **299**, 797-802 (1982).
25. Wilbur, W. J. & Lipman, D. J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 726-730 (1983).

Transient reversion of *ras* oncogene-induced cell transformation by antibodies specific for amino acid 12 of *ras* protein

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The proteins encoded by the *ras* oncogene are thought to trigger expression of the transformed phenotype in some types of cancer cells. In human cells, the *ras* protein family consists of several members including normal (proto-oncogene) and mutant (oncogene) forms[1-3]. In general, the proto-oncogene forms are thought to be involved in the normal growth control of cells, while the mutant forms (which apparently result from somatic mutation of the normal *ras* genes) appear to be responsible, in part, for the loss of normal growth control. On microinjection into living normal cells, the purified *ras* oncogene protein (p21) induces a characteristic loss of growth control in cells within several hours[4,5]. The mutant forms of the different *ras* proteins typically contain a single amino-acid change, usually at position 12 or less frequently at position 61 (ref. 6). Here we report that microinjection of antibodies specific for amino acid 12 of the oncogenic v-Ki-*ras* protein into cells transformed by this protein causes a transient reversion of the cells to a normal phenotype. The fact that this antibody inhibits binding of GTP to the v-Ki-*ras* protein supports the notion that GTP binding is essential to the transforming function of this oncogene product.

The role of *ras* proteins in cellular metabolism is not yet understood, but it is known that they are localized on the inner surface of the plasma membrane and bind GTP and GDP[7,8]. Recent analysis of the normal and mutant forms of the human Ha-*ras* proteins purified from bacterial expression systems[9,10] or present in the bacterial lysates after expression[11] has shown that *ras* proteins are able to hydrolyse GTP to GDP. It has been

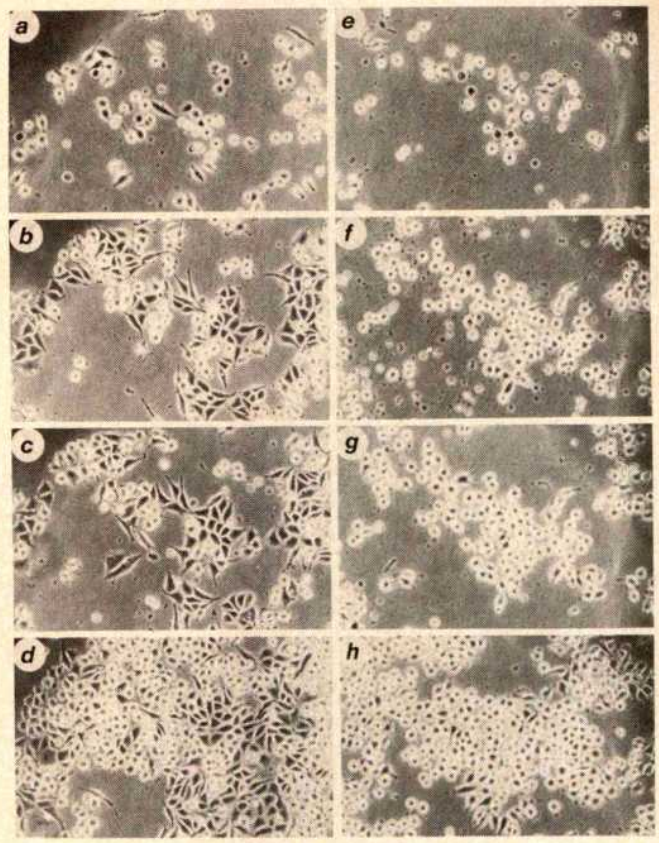


Fig. 1 Morphology of Ki-NRK cells after microinjection of anti-p21-Ser and control IgG. Approximately 35 Ki-NRK cells (growing in 10% fetal calf serum in Dulbecco's modified Eagle's medium) in each of the two test areas (marked by ink circles under the dish) were microinjected with anti-p21-Ser (*a-d*) or non-immune IgG (*e-h*) as described elsewhere[18]. Phase-contrast photomicrographs were taken of the two areas 30 min (*a, e*), 24 h (*b, f*), 36 h (*c, g*) and 48 h (*d, h*) after injection. Note that the cells injected with anti-p21-Ser show a normal flattened morphology for some time and grow at a slower rate than either the surrounding uninjected cells or the cells injected with the control IgG. ×4.

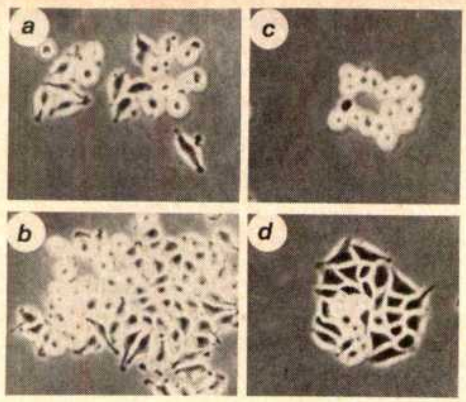


Fig. 2 Slower growth rate of Ki-NRK cells after microinjection of anti-p21-Ser antibody. Two areas of sparsely plated Ki-NRK cells growing on a dish (see Fig. 1 legend) were injected with either control IgG (*a*) or anti-p21-Ser IgG (*c*). The two areas were photographed immediately after injection (*a* and *c*) and 24 h later (*b* and *d*, respectively). Note the different rates of growth between the two areas of cells.

suggested that *ras* proteins function in a manner analogous to the 'G' regulatory protein of the adenylate cyclase system[4,9–13], which is also localized in the plasma membrane; this model is particularly attractive because G proteins activate adenylate cyclase through a GTP-dependent reaction, and inactivate the enzyme through hydrolysis of bound GTP to GDP and P_i. Position 12 mutants of human Ha-*ras* protein show a 10-fold reduction in rate of GTP hydrolysis[9–11], and may therefore represent abnormally activated regulatory proteins.

To obtain a biochemical probe capable of distinguishing between the normal and oncogenic forms of the *ras* proteins, antibodies were raised against *ras* (p21)-related peptides that differed at position 12. One antibody, designated anti-p21-Ser, was affinity-purified from the serum of rabbits immunized with the peptide ^{6}Lys-Leu-Val-Val-Gly-Ala-Ser-Gly-Val-Gly-Lys Cys-^{18}Ser (Ser at position 12) coupled covalently to carrier protein (R.C., G.W., D. Nitecki and F. McC., unpublished). Anti-p21-Ser was found to bind the v-Ki-*ras* protein (serine at position 12) but neither the v-Ha-*ras* protein (arginine at position 12) nor the *ras* protein containing glycine (normal) at position 12. In addition, biochemical studies showed that this antibody blocked both the GTP-dependent autophosphorylation and GTP-binding activities of the v-Ki-*ras* protein, suggesting that the antibody and GTP bind to the same region of the protein.

Given this high degree of specificity of the affinity-purified antibody preparation for the oncogenic v-Ki-*ras* protein, as well as the ability of the antibody to inhibit the GTP binding activity of the v-Ki-*ras* protein, it seemed possible that introduction of this antibody preparation into living, transformed cells might block the biochemical action of the *ras* oncogene protein *in vivo*, without affecting the function of the proto-oncogene product. Using microneedle injection procedures, $\sim 5 \times 10^5$ molecules of purified IgG (either anti-p21-Ser or non-immune serum) were injected into the cytoplasm of NRK cells transformed by the v-Ki-*ras* oncogene (called Ki-NRK cells); this represents a 5–10-fold excess of antibody over the estimated amount of *ras* protein in these cells[4]. Within 15 h and persisting for 48 h after injection of the anti-p21-Ser antibodies, the Ki-NRK cells showed a flattened, normal morphology (Fig. 1*a–d*); after 48 h, the cells reverted to their rounded morphology. In contrast, cells on the same dish which had been injected with control IgG showed no obvious change in morphology (Fig. 1*e–h*). (Control IgGs included goat anti-mouse IgG and rabbit non-immune IgG.) Inspection of the photographs taken for these experiments indicated that in addition to the reversion of the transformed phenotype, the flattened cells appeared to grow at a slower rate than either cells injected with control IgG or surrounding uninjected cells; this is evident from Fig. 2, where cells injected with control IgG have tripled in number over 24 h post-injection while cells injected with the anti-p21-Ser IgG have only doubled in number over the same time period. Based on data from the genetic analysis of the yeast *ras* genes which indicated that complete elimination of *ras* gene expression yielded non-viable cells[14,15], we might predict that antibodies that block the function of both the normal and oncogenic forms of the *ras* proteins would kill or prevent growth of microinjected transformed mammalian cells. Figures 1 and 2 show, however, that the cells injected with anti-p21-Ser are indeed able to grow, a result in agreement with biochemical experiments which showed that this antibody binds only to the oncogenic form of the *ras* protein with serine at position 12 (R.C., G.W., N.A., D. Nitecki and F.McC., unpublished).

By using immunofluorescence microscopy, we were able to determine exactly which cells had been injected with the two antibody preparations. Those cells that had been injected with

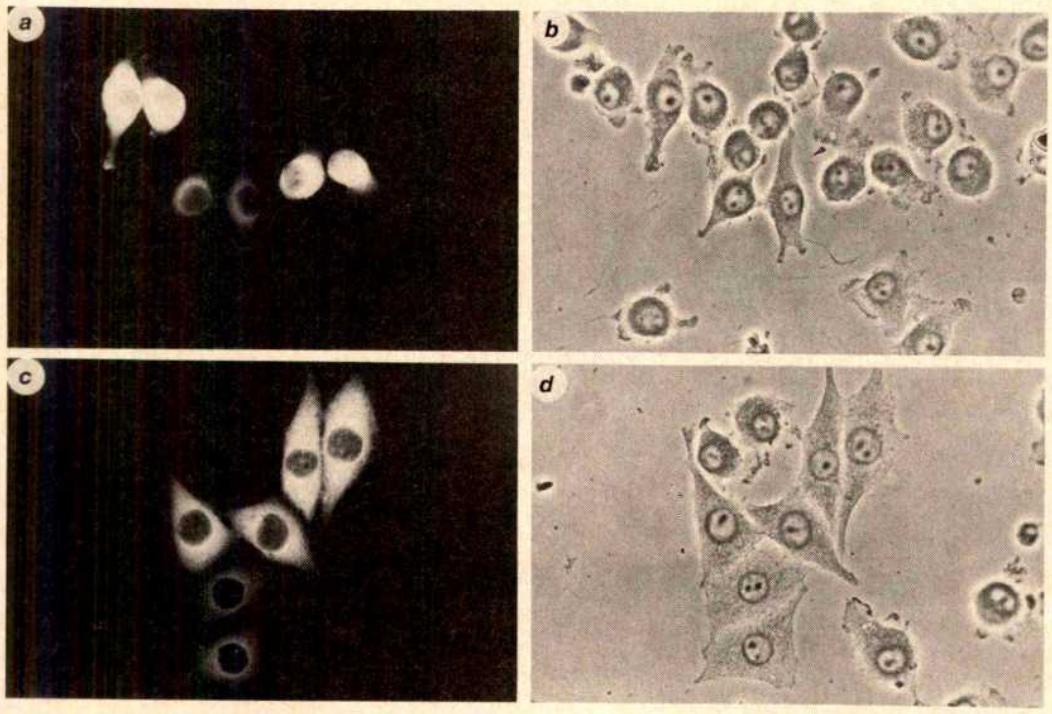


Fig. 3 Correspondence of the flattened appearance of Ki-NRK cells with microinjection of anti-p21-Ser antibodies. Ki-NRK cells (growing on glass coverslips) were injected with non-immune IgG (*a*, *b*) or anti-p21-Ser (*c*, *d*) and fixed (absolute methanol, −20 °C, 5 min) 24 h after injection. The cells were treated with fluorescein isothiocyanate-labelled goat anti-rabbit IgG (Cappel Laboratories), which stained only the injected cells. The fields of cells were photographed by epifluorescence (*a*, *c*) and phase-contrast (*b*, *d*) microscopy with a Zeiss Photomicroscope III as described elsewhere[18]. *a*, *b* Represent the same field of cells injected with control IgG and *c* and *d* the same field of cells injected with anti-p21-Ser.

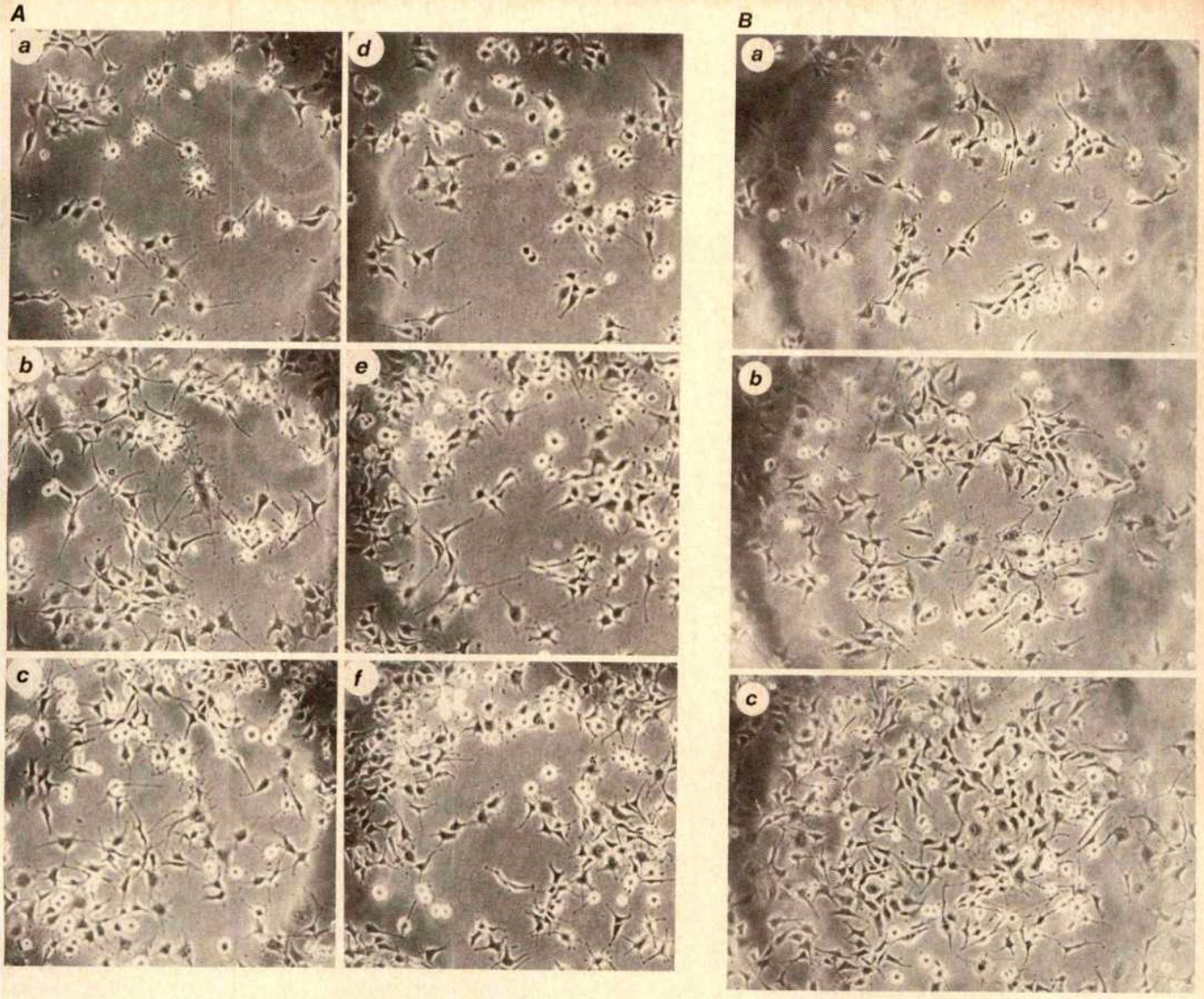


Fig. 4 Morphology of Ha-NRK cells after microinjection of: *A*, anti-p21-Ser and control IgG; and *B*, monoclonal antibody Y-238. *A*, Approximately 25 Ha-NRK cells in defined areas (marked by ink circles on the bottom of the dish) were injected with anti-p21-Ser (*a–c*) or non-immune IgG (*d–f*). The areas were photographed 30 min (*a*, *b*), 24 h (*c*, *d*) and 36 h (*e*, *f*) post-injection. No change in morphology or growth rate was observed for either antibody. *B*, Approximately 30 Ha-NRK cells were injected with monoclonal antibody Y-238 (ref. 16) and photographed 30 min (*a*), 24 h (*b*), and 36 h (*c*) after injection. Note the lack of effect of this monoclonal antibody on either the morphology or the growth rate of the cells.

anti-p21-Ser had the morphologically normal, flattened appearance (Fig. 3*c*, *d*) whereas cells injected with control IgG retained their rounded, transformed appearance (Fig. 3*a*, *b*).

To determine the specificity of the *in vivo* effect of the anti-p21-Ser preparation in terms of cells transformed by other oncogenic *ras* proteins, the anti-p21-Ser and control IgGs were injected (5×10^5 molecules per cell) into Ha-NRK cells (that is, NRK cells transformed by the v-Ha-*ras* oncogene protein, which has an arginine at position 12). Photomicroscopy indicated that neither antibody had an effect on these cells (Fig. 4*A*); this result is consistent with the observation that, *in vitro*, the anti-p21-Ser preparation does not react with the v-Ha-*ras* protein (R.C., G.W., N.A., D. Nitecki and F.McC., unpublished).

To determine whether the ability of anti-p21-Ser to induce reversion to normal phenotype is a general property of anti-p21 antibodies, one other antibody was tested by this assay. Furth and co-workers have produced several rat-derived monoclonal antibodies against the v-Ha-*ras* protein[16], including one that is relatively specific for the Ha-*ras* proteins (called Y-238); this antibody does not block GTP binding activity, does not discriminate between normal and mutant forms of the *ras* protein and probably binds to a site in the Ha-*ras* protein that is not influenced by amino acid 12 of the protein. Y-238 was microinjected into Ha-NRK cells (1×10^6 molecules per cell), and the cells were examined by photomicroscopy for 48 h. Neither the Y-238 monoclonal antibody nor a control IgG preparation had any effect on the morphology or growth rate of the cells (Fig. 4*B*); similar results were obtained using Y-259 anti-p21 antibody. Thus, the antibody either binds to a non-essential site on the *ras* protein or does not bind at all to the *ras* protein in these cells.

These results, together with the ability of anti-p21-Ser to block GTP binding by p21 *in vitro* (R.C., G.W., N.A., D. Nitecki and F.McC., unpublished), are consistent with the idea that the interaction of p21 with GTP is crucial to the transforming function of the *ras* gene. However, further studies are required to examine the possibility that the antibody disrupts other important activities of p21 such as membrane localization or interactions with cellular proteins. Whatever mechanism is responsible for the observed effects of the microinjected anti-p21-Ser on cell behaviour, the data presented here indicate that antibodies capable of inactivating oncogene but not proto-oncogene products can block the expression of the transformed phenotype in living cells. Gallick *et al.*, using antibodies directed against the $p37^{mos}$ oncogene product of the Moloney murine sarcoma virus, reached a similar conclusion; microinjection of the antibodies yielded at least a different morphology in transformed cells[17].

When antibodies specific for other single mutations in the *ras* protein family (N.A. *et al.*, in preparation) become available, we will be able to extend this approach to inhibition of the function of the other forms of the oncogenic *ras* proteins in living cell and biochemical systems. Such experiments, coupled with studies in which the human *ras* oncogene protein is injected into normal cells, resulting in transient proliferation[4,5], provide new ways of examining the physiology and cell biochemistry of the transformation process.

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1. Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3637–3640 (1982).
2. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297**, 474–478 (1982).
3. Shimizu, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 2112–2116 (1983).
4. Feramisco, J. R., Kamata, T., Gross, M., Rosenberg, M. & Sweet, R. W. *Cell* **38**, 109–117 (1984).
5. Stacey, D. & Kung, H. *Nature* **310**, 508–511 (1984).
6. Marshall, C. J. & Rigby, P. W. J. *Cancer Surv.* **3**, 183–214 (1984).
7. Willingham, M. C., Partan, I., Shih, T. Y. & Scolnick, E. M. *Cell* **19**, 1005–1014 (1980).
8. Shih, T. Y., Papageorge, A. G., Stoker, P. E., Weeks, M. O. & Scolnick, E. M. *Nature* **287**, 686–691 (1980).
9. Sweet, R. W. *et al. Nature* **311**, 273–275 (1984).
10. Gibbs, J. B., Sigal, I. S., Poe, M. & Scolnick, E. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 5704–5708 (1984).
11. McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. *Nature* **310**, 644–649 (1984).
12. Gilman, A. G. *Cell* **36**, 577–579 (1984).
13. Kamata, T. & Feramisco, J. R. *Cancer Cells* **1**, 11–16 (1984).
14. Tatchell, K., Chaleff, D., Defeo-Jones, D. & Scolnick, E. *Nature* **309**, 523–527 (1984).
15. Kataoka, T. *et al. Cell* **37**, 437–455 (1984).
16. Furth, M. E., Davis, L. J., Fleurdelys, B. & Scolnick, E. M. *J. Virol.* **43**, 294–304 (1982).
17. Gallick, G. E., Brown, D. B., Murphy, E. C. Jr & Arlinghaus, R. B. in *Modern Approaches to Vaccines* (eds Chanock, R. M. & Lerner, R. A.) 407–413 (Cold Spring Harbor Laboratory, New York).
18. Wang, K., Feramisco, J. R. & Ash, J. F. *Meth. Enzym.* **85**, 514–562 (1982).

Production of 'hybrid' antibiotics by genetic engineering

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The recent development of molecular cloning systems in *Streptomyces*[1–4] has made possible the isolation of biosynthetic genes for some of the many antibiotics produced by members of this important genus of bacteria[5–10]. Such clones can now be used to test the idea that novel antibiotics could arise through the transfer of biosynthetic genes between streptomycetes producing different antibiotics[11]. The likelihood of a 'hybrid' compound being produced must depend on the substrate specificities of the biosynthetic enzymes, about which little is known. In attempts to demonstrate hybrid antibiotic production, we therefore began with strains producing different members of the same chemical class of compounds in order to maximize the chance of success. Here we report the production of novel compounds by gene transfer between strains producing the isochromanequinone antibiotics actinorhodin[12], granaticin[13] and medermycin[14]. These experiments were made possible by the recent cloning of the whole set of genes for the biosynthetic pathway of actinorhodin from *Streptomyces coelicolor* A3(2) (ref. 8). We believe that this represents the first report of the production of hybrid antibiotics by genetic engineering.

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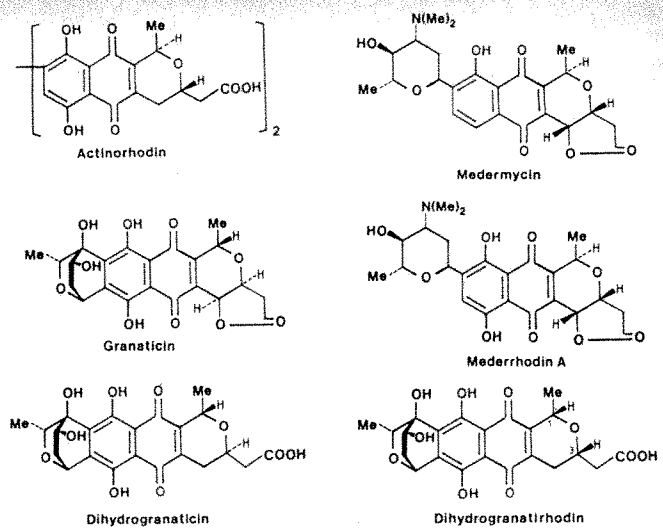


Fig. 1 Structures of the isochromanequinone antibiotics. The absolute stereochemistry of dihydrogranatirhodin is tentative (see text).

Plasmid pIJ2303 (ref. 8) consists of the low-copy-number vector plasmid pIJ922, derived from SCP2* (ref. 15), with 32.5 kilobases (kb) of *S. coelicolor* A3(2) DNA inserted at its unique *Bam*HI site[8]. That this inserted DNA carries the complete cluster of genes (*act*) for the biosynthesis of actinorhodin (Fig. 1) from simple primary metabolites (probably acetate) is shown by complementation of all available classes of *S. coelicolor act* mutants by pIJ2303 and by the production of actinorhodin by several species of *Streptomyces* (including *S. parvulus* ATCC 12434, *S. peucetius* ATCC 29050, *S. tanashiensis* KA-415 and *S. glaucescens* ETH 22794) carrying pIJ2303 (F.M., H.M.K. and D.A.H., unpublished).

In an analysis of the organization and expression of the *act* genes, segments of the total *act* DNA of pIJ2303 have recently been inserted (F.M., unpublished) into the *Bam*HI site of pIJ922 or into the *Bam*HI, *Bgl*II or *Pst*I sites of pIJ940 (a second low-copy-number vector derived from SCP2*; ref. 15). Some of these subclones, carrying different transcription units of the *act* region, were introduced by transformation into *Streptomyces* sp. AM-7161 (the producer of medermycin), *Streptomyces violaceoruber* Tü22 (the producer of granaticin), and a mutant (B1140) of Tü22 blocked in granaticin synthesis, by selecting for the thiostrepton-resistance marker of the vectors. The efficiency of transformation of these cultures, using standard conditions developed for *Streptomyces lividans* and *S. coelicolor*[16], by DNA of the clones (isolated from *S. coelicolor* A3(2) derivatives) was only 1–10 per μg of plasmid DNA, compared with 10^6–10^7 in *S. coelicolor* A3(2) (ref. 8). These reduced frequencies may reflect the operation of restriction systems, as well as the fact that no attempt was made to optimize transformation conditions for these strains. Nevertheless, transformants were obtained in all of the combinations attempted (Table 1).

Actinorhodin, granaticin and medermycin are all acid–base indicators which confer characteristic colours on the cultures producing them: red↔blue for actinorhodin; red↔purple for granaticin; and yellow↔brown for medermycin. It was apparent from the colours of AM-7161 and B1140 carrying pIJ2303 that the *S. coelicolor act* genes were being expressed in the recipient strains, as blue pigments were present in alkaline conditions. More strikingly, the colours of the cultures produced by introducing certain of the partial *act* clones into the medermycin producer (AM-7161) indicated that one or more novel compounds were present. Notable was the bright purple colour, at

Table 1 Antibiotics produced by the *Streptomyces* strains

Strain	Colour of culture‡		Relevant compounds identified†
	Acidic	Alkaline	
S. coelicolor A3(2)	Red	Blue	Actinorhodin
Streptomyces sp. AM-7161	Yellow	Brown	Medermycin
AM-7161/pIJ2303*	Red	Blue	Medermycin, actinorhodin
AM-7161/pIJ2301*	Red	Purple	Mederrhodin A, medermycin
AM-7161/pIJ2304	Yellow	Brown	Medermycin
AM-7161/pIJ2308	Yellow	Brown	NT
AM-7161/pIJ2312	Yellow	Brown	NT
AM-7161/pIJ2315*	Red	Purple	Mederrhodin A, mederrhodin B, medermycin
AM-7161/pIJ2316*	Red	Purple	Mederrhodin A, medermycin
AM-7161/pIJ2317	Yellow	Brown	NT
S. violaceoruber Tü22	Red	Blue-purple	Granaticin, dihydrogranaticin
Tü22/pIJ2303*	Red	Blue-purple	Dihydrogranatirhodin, actinorhodin
S. violaceoruber B1140	Yellow	Brown	NT
B1140/pIJ2303	Red	Blue-purple	Granaticin, dihydrogranaticin, actinorhodin
B1140/pIJ2301	Yellow	Brown	NT
B1140/pIJ2302	Yellow	Brown	NT
B1140/pIJ2304	Yellow	Brown	NT
B1140/pIJ2308*	Red	Blue-purple	Granaticin, dihydrogranaticin
B1140/pIJ2310	Yellow	Brown	NT
B1140/pIJ2311	Yellow	Brown	NT
B1140/pIJ2314	Yellow	Brown	NT

* Plasmid DNA from these cultures was shown to be indistinguishable in structure from the same plasmid originally isolated in *S. coelicolor*, as judged by *Bam*HI digestion.

† To characterize the pigments present in AM-7161 carrying various clones, cultures were grown in liquid medium (seed stage: 2% glucose, 0.3% peptone, 0.3% beef extract, 0.1% yeast extract, 0.3% NaCl, 0.1% $CaCO_3$, *p*H 7.0, for 48 h at 28 °C; production stage: 1.5% glycerol, 1% soybean meal, 0.3% NaCl, *p*H 7.0, with shaking for 96 h at 28 °C, after addition of a 10% inoculum from the seed stage). The broth was adjusted to *p*H 7.0 with 1 M HCl and extracted with chloroform. After concentration of the extracts, they were analysed by silica-gel TLC run in chloroform/methanol (5:1). The pigments produced by cultures of Tü22 carrying pIJ2303, and B1140 carrying pIJ2303 and pIJ2308, were obtained from fermentations in a medium containing 1% raw ground peanuts, 1% glucose, 0.5% NaCl, 0.35% $CaCO_3$, 70 mg l^{-1} histidine, 10 mg l^{-1} guanine, 15 mg l^{-1} thiostrepton in baffled flasks shaken at 300 r.p.m. at 28 °C. Seed cultures were grown for 24 h and production cultures for 48 h (granaticin-related pigments) or 144 h (actinorhodin) after addition of 5% inoculum. The broth was then adjusted to *p*H 2.0 and the culture was filtered. The residue was resuspended in 1 M NaOH and re-filtered. After adjusting the *p*H of the filtrate to 2.0, the precipitated crude pigments were collected by centrifugation and dried under vacuum. TLC analyses were done on oxalic acid-impregnated silica gel (chloroform/ethyl acetate, 6:4).

‡ Cultures were grown on R2YE plates[16] (containing histidine and guanine in the case of B1140, which carries two auxotrophic mutations); after 4–5 days they were exposed to the fumes of either concentrated HCl or ammonia solution. NT, not tested.

alkaline *p*H, of the culture carrying pIJ2315; cultures carrying pIJ2301 and pIJ2316 had a duller purple colour. B1140 carrying pIJ2308 had a colour resembling that of granaticin, and this result also demonstrated a 'cooperation' between gene products in the actinorhodin and granaticin pathways.

We characterized the pigments present in AM-7161 carrying various clones. The results (Table 1) confirmed that AM-7161 carrying pIJ2315 produced, in addition to medermycin, large amounts of a novel compound; this material was also present, in smaller quantities, in AM-7161 carrying pIJ2301 and pIJ2316, and was termed 'mederrhodin A'. A second novel compound ('mederrhodin B') was present in the aqueous phase after chloroform extraction of the AM-7161/pIJ2315 broth. Interestingly, AM-7161 carrying pIJ2303 produced abundant actinorhodin, together with medermycin, but no novel compounds were detected.

Mederrhodin A was purified by passing the acidified broth of AM-7161/pIJ2315 through a Diaion HP-20 column (Mitsubishi) to adsorb medermycin and related compounds. The column was washed with deionized water and the pigments were eluted with 70% aqueous acetone. After evaporation of the acetone from appropriate fractions, the concentrate was adjusted to *p*H 7.0 with 1M NaOH and extracted twice with equal volumes of chloroform. Mederrhodin A was then purified by preparative TLC. Structural determination of field desorption-mass spectroscopy and element analysis revealed hydroxymedermycin, and the position of the hydroxyl group was determined by ultraviolet and ^{1}H-NMR spectroscopy (Fig. 1). Mederrhodin A, like medermycin, was found to have antibiotic activity against various Gram-positive bacteria.

The pigments produced by cultures of Tü22 carrying pIJ2303, and B1140 carrying pIJ2303 and pIJ2308, were obtained as described in Table 1. B1140/pIJ2303 and B1140/pIJ2308 produced a mixture of granaticin and dihydrogranaticin (Fig. 1), pigments characteristic of the Tü22 strain; actinorhodin was also present in B1140/pIJ2303, but no novel compounds were detected. However, Tü22 carrying pIJ2303 produced, in addition to actinorhodin, a novel compound related to dihydrogranaticin; this compound was named 'dihydrogranatirhodin'. Interestingly, very little granaticin or dihydrogranaticin was present.

Dihydrogranatirhodin was purified by column chromatography on oxalic acid-treated silica gel (chloroform) and preparative TLC, and was converted to the methyl ester as described for dihydrogranaticin[17]. The mass spectrum gave the molecular formula $C_{23}H_{24}O_{10}$ and the ultraviolet/visible spectrum indicated the presence of the same 5,8-dihydroxy-1,4-naphthoquinone chromophore as in dihydrogranaticin. The ^{1}H-NMR spectrum closely resembles that of dihydrogranaticin, but shows up-field shifts of the signals for H-3 and H-1, as has been seen in synthetic model compounds with *cis*-arrangement of these two hydrogens[17]. This was confirmed by observation of a nuclear Overhauser enhancement of 5.5% for H-3 on irradiation of H-1. Hence, dihydrogranatirhodin is either the C-3 or the C-1 epimer of dihydrogranaticin. As the configuration at C-3 in actinorhodin is determined early in the biosynthesis by reduction of the C-3 carbonyl group (X.G.He, S. P. Cole and H.G.F., unpublished), we suggest that dihydrogranatirhodin has the actinorhodin (3S) configuration at C-3 and the granaticin (1S) configuration at C-1 (Fig. 1).

The demonstration of novel compounds in hybrid strains prepared by transferring actinorhodin genes into both the granaticin- and the medermycin-producer augurs well for the widespread usefulness of genetic engineering as a new tool in antibiotic discovery. There seems little doubt that dihydrogranatirhodin and the mederrhodins are indeed the products of 'hybrid' biosynthetic pathways arising through an interaction

between enzymes encoded by structural genes originating from two different streptomycetes, rather than the results of the activation of latent genetic information in the recipient strains. (The latter mechanism is the most probable explanation for the three reports of the discovery of novel compounds through natural inter-strain matings (refs 18–20; discussed in ref. 21).) The evidence is most compelling for mederrhodin A, since, of the clones tested, pIJ2301, pIJ2315 and pIJ2316 (which led to mederrhodin A synthesis by AM-7161) all contain a complete transcription unit (absent from pIJ2304, pIJ2308, pIJ2312 and pIJ2317) which complements class V *act* mutants of *S. coelicolor* (F.M., unpublished); such mutants have recently been shown to be blocked in the corresponding hydroxylation involved in actinorhodin biosynthesis (S. P. Cole and H.G.F., unpublished). A true metabolic cooperation between the actinorhodin and granaticin biosynthetic enzymes is indicated also by complementation of the B1140 mutant by pIJ2308, but not by the other clones tested. B1140 can act as a secretor in co-synthesis tests with *S. coelicolor act* mutants of classes I, III and VII, but not of classes IV, V and VI[22], suggesting that its block is equivalent to that of class IV *act* mutants. Significantly, pIJ2308 is the only clone tested that carries a complete transcription unit for the class IV *act* gene (F.M., unpublished). Moreover, the 'mixed' stereochemistry found in dihydrogranatirhodin has not previously been encountered in any isochromanequinone antibiotic, even though a strain of *Streptomyces roseofulvus* has recently been found to produce both frenolicin B (with the same configuration of the pyranoid ring as actinorhodin) and nanaomycins A and βA [with the same configuration as granaticin (S.Ō. *et al.*, unpublished)].

The different results obtained with AM-7161 and Tü22 carrying the complete set of actinorhodin biosynthetic genes is interesting. In AM-7161, the donor and recipient gene sets appear to operate independently, with production of the two parental antibiotics but no novel compounds. Only when a partial *act* clone was introduced into the medermycin-producer was a hybrid product formed. In contrast, Tü22 carrying pIJ2303 produced dihydrogranatirhodin (as well as some actinorhodin), to the almost total exclusion of the normal antibiotics of the recipient. This differing behaviour may reflect several factors, including the relative specificities and affinities of the biosynthetic enzymes for their substrates, the regulation of gene expression and any possible 'channelling' of precursors within individual biosynthetic pathways. Such considerations must be borne in mind in the future use of genetic engineering in antibiotic discovery, or in attempts to modify existing antibiotics in predetermined ways.

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1. Bibb, M. J., Schottel, J. L. & Cohen, S. N. *Nature* **284,** 526–531 (1980).
2. Suarez, J. E. & Chater, K. F. *Nature* **286,** 527–529 (1980).
3. Thompson, C. J., Ward, J. M. & Hopwood, D. A. *Nature* **286,** 525–527 (1980).
4. Bibb, M. J., Chater, K. F. & Hopwood, D. A. in *Experimental Manipulation of Gene Expression* (ed. Inouye, M.) 53–82 (Academic, New York, 1983).
5. Feitelson, J. S. & Hopwood, D. A. *Molec. gen. Genet.* **190,** 394–398 (1983).
6. Gil, J. A. & Hopwood, D. A. *Gene* **25,** 119–132 (1983).
7. Chater, K. F. & Bruton, C. J. *Gene* **26,** 67–78 (1983).
8. Malpartida, F. & Hopwood, D. A. *Nature* **309,** 462–464 (1984).
9. Bailey, C. R., Butler, M. J., Normansell, N. D., Rowlands, R. T. & Winstanley, D. J. *Bio/Technology* **2,** 808–811 (1984).
10. Jones, G. H. & Hopwood, D. A. *J. biol. Chem.* **259,** 14151–14157 (1984).
11. Hopwood, D. A. in *Beta-lactam Antibiotics* (eds Salton, M. R. J. & Shockmann, G. D.) 585–598 (Academic, New York, 1981).
12. Brockmann, H., Zeeck, A., van der Merve, K. & Müller, W. *Justus Liebigs Annln Chem.* **698,** 3575–3579 (1966).
13. Carbaz, R. *et al. Helv. chim. Acta* **40,** 1262–1269 (1957).
14. Ogura, H. & Furuhata, K. *Abstr. 9th int. Congr. Heterocyclic Chem.*, 114 (1983).
15. Lydiate, D. J., Malpartida, F. & Hopwood, D. A. *Gene* (in the press).
16. Thompson, C. J., Ward, J. M. & Hopwood, D. A. *J. Bact.* **151,** 668–677 (1982).
17. Pyrek, J. S.-P., Achmatowicz, O. & Zamojski, A. *Tetrahedron* **33,** 673–680 (1977).
18. Ihn, W., Schlegel, B., Fleck, W. F. & Sedmera, P. *J. Antibiot., Tokyo* **33,** 1457–1461 (1980).
19. Mazières, N., Peyré, M. & Pénasse, L. *J. Antibiot., Tokyo* **34,** 544–550 (1981).
20. Traxler, P., Schupp, T., Fuhrer, H. & Richter, W. J. *J. Antibiot., Tokyo* **34,** 971–979 (1981).
21. Hopwood, D. A. in *Biochemistry and Genetic Regulation of Commercially Important Antibiotics* (ed. Vining, L. C.) 1–23 (Addison-Wesley, Reading, Massachusetts, 1983).
22. Rudd, B. A. M. & Hopwood, D. A. *J. gen. Microbiol.* **114,** 35–43 (1979).

Molecular cloning of Ancient Egyptian mummy DNA

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Artificial mummification was practised in Egypt from ~2600 BC until the fourth century AD. Because of the dry Egyptian climate, however, there are also many natural mummies preserved from earlier as well as later times. To elucidate whether this unique source of ancient human remains can be used for molecular genetic analyses, 23 mummies were investigated for DNA content. One 2,400-yr-old mummy of a child was found to contain DNA that could be molecularly cloned in a plasmid vector. I report here that one such clone contains two members of the *Alu* family of human repetitive DNA sequences, as detected by DNA hybridizations and nucleotide sequencing. These analyses show that substantial pieces of mummy DNA (3.4 kilobases) can be cloned and that the DNA fragments seem to contain little or no modifications introduced postmortem.

Samples were removed from 23 different mummies and mummy fragments. These specimens varied in age from the Sixth Dynasty (~2370–2160 BC) to late Roman times. Whenever possible, representative samples were taken from all the different tissues that could be identified visually. After rehydration and preparation of microscopical sections[1], the samples were studied using conventional histological stains as well as staining by ethidium bromide, which allows detection of small amounts of DNA. The cartilage cells from the outer ear of a mummified female head (Egyptian Museum, Berlin, GDR) as well as cells in the epidermis and subcutaneous tissues of a male head (Viktoria Museum, Uppsala; inventory number VM3251) proved to contain identifiable cell nuclei stainable by ethidium bromide. When the DNA from the latter sample was extracted, however, it was found to contain modified pyrimidines[2,3] and was intractable to molecular cloning. In contrast, cells of the epidermis and several subcutaneous structures from a less than 1-yr-old boy from the collections of the Egyptian Museum,

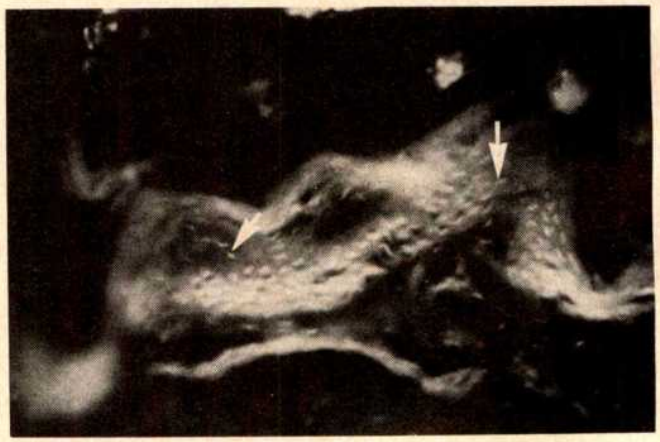


Fig. 1 Tissue section of skin from the left lower leg of the Berlin mummy used for molecular DNA cloning. Ethidium bromide staining allows the visualization of nucleic acids in the cell nuclei (arrows).
Methods. Small tissue samples were rehydrated in an aqueous solution of 1% Na_2CO_3 (w/v), 0.5% formalin and 28.5% ethanol[1] for 48 h. Paraffin embedding and sectioning were performed using routine protocols. After deparaffination, the sections were stained in a 5 $\mu g\ ml^{-1}$ solution of ethidium bromide in phosphate-buffered saline (PBS) for 30 min, followed by extensive washing in PBS and inspection under a Leitz fluorescence microscope. ×180.

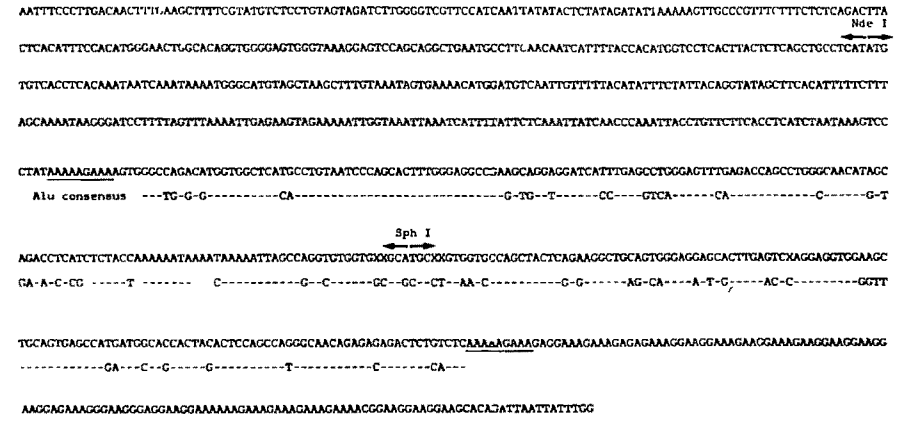


Fig. 2 DNA sequence of part of the mummy clone pMUM2:9. The 9-bp direct repeats flanking the *Alu* sequence are underlined. In the *Alu* consensus sequence[8] only nucleotides differing from the pMUM2:9 sequence are specified

Methods. DNA was prepared from 1.6 g mummy tissue, essentially according to the protocol described previously[5]. The DNA was made blunt-ended with *Escherichia coli* DNA polymerase (Klenow fragment) (Pharmacia P-L Biochemicals) according to the manufacturer's instructions and in the presence of trace amounts of radioactively labelled nucleotides The sample was then size-fractionated by gel filtration on a G-50 column (Pharmacia, Uppsala) Excluded fractions were pooled and ethanol-precipitated and 25 ng of this material cloned in a *Sma*I-digested and alkaline phosphatase-treated pUC8 plasmid[6]. Then, 700 of the white clones were transferred to nitrocellulose filters and screened[20] with a nick-translated[7] 550-bp *Bgl*II/*Sph*I fragment from a HLA-DR pseudogene[21], which contains an *Alu* repeat. The strongly hybridizing clone pMUM2:9 was isolated and restriction-mapped. Two *Alu* repeats were identified by Southern hybridization[9]. One of the *Alu* repeats as well as 500 bp of flanking DNA were sequenced according to the Maxam and Gilbert procedure[22] after labelling of the *Sph*I and *Nde*I restriction sites indicated.

Berlin, GDR (inventory no. 721) were preserved excellently by the above-mentioned criteria (Fig. 1). The radiocarbon age of this mummy was determined to 2,430 ± 120 BP, by the technique of accelerator mass spectroscopy[4].

I removed 1.6 g dry tissue from superficial parts of the left lower leg of the mummy and extracted DNA using detergent and sodium perchlorate[5] followed by phenol extraction and ethanol precipitation. When analysed by agarose gel electrophoresis, most of the DNA was <500 base pairs (bp) long. A minor portion of the material, however, was clearly ≥5,000 bp long (data not shown). Next, 15% of the extracted material was blunt-ended with *Escherichia coli* DNA polymerase (Klenow fragment) in the presence of trace amounts of radioactively labelled nucleotides. After termination of the reaction, the sample was size-fractionated by gel filtration and fractions containing the larger DNA fragments pooled and precipitated with ethanol. Then, 0.5% of this DNA was cloned in the bacterial plasmid pUC8 (ref. 6) and ~1,000 clones were obtained, 700 of which were transferred to nitrocellulose filters. Replicas of these were screened by colony hybridization using a radioactively labelled probe[7] containing a member of the *Alu* family of repetitive human sequences. The *Alu* repeats are present in at least 300,000 copies per human genome[8]. One clone (pMUM2:9), with an insert of 3.4 kilobases (kb), was identified and subjected to further analysis. On mapping by Southern hybridization[9], the insert of this clone was found to contain two different segments hybridizing to the *Alu* probe. I sequenced one of these segments and 500 bp of flanking DNA (Fig. 2). The *Alu* repeat displays 77% homology to a human *Alu* consensus sequence[8] and is flanked by 9-bp-long direct repeats. On its 3′ side it is followed immediately by a dAMP-rich region of almost 100 nucleotides. Of the point mutations present in the sequence, only 30% represent transversions, in agreement with the types of divergence found among *Alu* repeats[10]. Therefore, no significant postmortem modifications seem to have affected the nucleotide sequences analysed, even if chemical sequence alterations affecting a very small proportion of the nucleotides cannot be excluded. The DNA sequences flanking the *Alu* repeat show no statistically significant homology to any known DNA sequences contained in a DNA sequence bank[11].

These results establish the feasibility of faithfully cloning substantial pieces of genomic DNA from biological remains of great antiquity. The yield of DNA from this unusually well-preserved mummy was ~20 μg per g dried tissue, which approaches 5% of the amount expected from fresh tissue. Note, however, that most of the mummy samples investigated seem to be devoid of nucleic acids. Interestingly, the general histological preservation of the various mummy tissues was much better in superficial tissues and peripheral parts of the bodies than in the more deeply situated tissues, an observation made also by others[12,13]. This probably results from the fact that the mummification process in Ancient Egypt consisted of dehydration of the eviscerated body by embedding it in crystalline salts (natron)[14]. Thus, the parts of the body in direct contact with the dehydrating agent may have dried out first, thus shortening the time that hydrolytic processes acted upon macromolecules in these tissues. Future attempts to clone genes from Ancient Egyptian human remains should therefore be directed towards superficial tissues.

The present report, as well as the recent cloning of mitochondrial DNA sequences from the dried skin of a quagga, extinct for ~100 years[15], raise the hope that recombinant DNA techniques may be applied systematically to archaeological samples. Thus, Ancient Egyptian mummies represent a unique material that could allow diachronic studies of the frequencies of polymorphic genes in the Egyptian population since neolithic times, as well as study of the evolution of DNA-containing viruses over the same time period. Finally, a number of Egyptological problems can now be approached, such as the descent of the ancient population in the Nile valley (see ref. 16 for review) and relationships between Pharaonic dynasties as well as individual relationships between members of the Pharaonic families[17–19].

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1 Sandtson, A T *Stain Technol.* **30**, 277–283 (1955)
2 Pääbo, S *Das Altertum* **30**, 213–218 (1984)
3 Paäbo, S *J archaeol Sci.* (submitted)
4 Possnert, G *Nucl. Instrum. Meth Phys Res* **B5**, 159–161 (1984)
5 Graham, D E *Analyt Biochem.* **85**, 609–613 (1978)
6 Vieira, J & Messing, J *Gene* **19**, 259–268 (1982)
7 Rigby, P W J, Dieckmann, M, Rhodes, C & Berg, P *J molec. Biol.* **113**, 237–251 (1977)
8 Schmid, C W & Jelinek, W R. *Science* **216**, 1065–1070 (1982)
9 Southern, E M *J molec. Biol* **98**, 504–517 (1975).
10 Deininger, P L, Jolly, D J, Rubin, C M, Friedmann, T & Schmid, C W *J molec Biol.* **151**, 17–33 (1981)
11 Barker, W C *et al Nucleic Acid Sequence Database* Version 22, National Biomedical Research Foundation (Washington, DC, 1984)
12 Daniels, F Jr & Post, P W. *Adv. Biol Skin* **10**, 279–292 (1970)
13 Chapel, T A, Mehregan, A H & Reyman, T A *J Am. Acad. Derm.* **4**, 27–30 (1981)
14 Lucas, A J *Egyptol Archaeol* **18**, 125–140 (1932)
15 Higuchi, R, Bowman, B, Freiberger, M, Ryder, O A & Wilson, A C *Nature* **312**, 282–284 (1984)
16 Trigger, B G. *The Cambridge History of Africa* (eds Fage, J D & Oliver, R) 478–547 (Cambridge University Press, 1982)
17 Harrison, R G, Connolly, R C. & Abdalla, A *Nature* **224**, 325–326 (1969)
18 Reeves, C N *Gottinger Miszellen* **54**, 61–71 (1982)
19 Harris, J E *et al Science* **200**, 1149–1151 (1978)
20 Gerger, J P, Stern, R H & Wensink, P C *Nucleic Acids Res* **7**, 2115–2136 (1979)
21 Larhammar, D, Servenius, B, Rask, L & Peterson, P A *Proc. natn Acad Sci. USA* (in the press)
22 Maxam, A M & Gilbert, W *Meth Enzym* **65**, 499–560 (1980)

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Edited by J. G. Scaife, D. Leach and A. Galizzi

1985, 272pp, $59.00/£45.00 (UK only), ISBN 0.12 621180.9

The book covers all the major genetic aspects of bacteria, their plasmids, and phages mutation· gene transfer and recombination, the transposition of genetic elements, and the mechanism and control of gene expression. Special emphasis is placed on *Escherichia coli*. In addition, *Bacillus subtilis*, a Gram-positive organism which has unique features as a genetic system, is introduced. For those wishing to study other bacteria a list of helpful references is provided at the end of Chapter One

ECOTOXICOLOGY

The Study of Pollutants in Ecosystems

F. Moriarty

1983, 240pp., $35 00/£22 00 (UK only), ISBN. 0.12.504760.7

The effects of pollutants on both ecosystems and individuals depend on the exposure to, and dose of, the pollutant, but in ecotoxicology, and distinct from toxicology, the significant effect is measured on species populations, not on individual organisms Failure to grasp this distinction has led to false conclusions, and the significance of much relevant ecological knowledge has been missed. This book has been written to remedy this shortcoming.

COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY OF ENZYMATIC DIGESTION

H. J. Vonk and J. R. H. Western

1984, 512pp, $80.00/£55 00 (UK only), ISBN· 0,12 727850.8

The ability of animals to consume and digest a wide range of organisms and organic materials is well established and a considerable literature has accumulated. Whereas most accounts concentrate on one animal or group of animals only, this book sets out to collate details of the digestive enzymes from the spectrum of metazoan animals and to facilitate comparison of the enzymes and digestive mechanisms in the different groups. Conclusions are given at the end of each chapter

ENZYME NOMENCLATURE

1984, 672pp., $45.00/£36 00 (UK only), ISBN· 0 12 227162 9 (Cloth)
1984, 672pp., $19 50/£16 00 (UK only), ISBN 0.12.227163 7 (Paper)

Recommendations of the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzyme-Catalysed Reactions. This edition is a revision of the Recommendations (1978) of the Nomenclature Committee of IUB, and has been approved for publication by the Executive Committee of the International Union of Biochemistry.

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Edited by Ion Gresser

1985, In Preparation, ISBN. 0.12.302255.X

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Written and Translated by **J. Moutschen,** *University de Liege, Belgium*
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Research in the market-place

Dorothy Nelkin

The Interferon Crusade: Public Policy and Biomedical Dreams.
By Sandra Panem.
The Brookings Institution, 1775 Massachusetts Avenue NW, Washington DC/ Costello, 43 The High Street, Tunbridge Wells, Kent: 1984. Pp. 109. Hbk $22.95, £31; pbk $8.95, £12.10.

"A MAGIC bullet", "a priceless miracle drug", "a cure for cancer", "a wonder therapy" — it was in these terms that the public was exposed to research on interferon. Interferon has been the most visible and dramatic product of the rapidly expanding biotechnology industry. The story of its development and the hyperbole that surrounded it reflect the mix of scientific, political and economic events that has generally shaped the biotechnology revolution. It is a story of promises and problems, of complex arrangements between government, industry and academia, and of the policy dilemmas of a fast-moving and potentially profitable field of research.

Sandra Panem's book, *The Interferon Crusade*, begins with the discovery in the 1950s that the body produces a natural therapeutic agent, "an interfering proton" to inhibit infections. Panem traces the development of interferon, documenting the discovery of its anti-tumour activity in 1967, the publicity about its potential as a cancer cure in the mid-1970s, and finally the successful cloning of a human interferon gene in late 1979. She writes also about the role of advocacy scientists, those key individuals who publicize research, capitalizing on the public fear of cancer in order to raise funds.

As Panem observes, the explosion of interest in interferon resulted mainly from its promise as a cure for cancer. The ability to synthesize the drug through genetic engineering techniques, and therefore to produce it inexpensively, aroused commercial attention so that interferon became the primary lure for trapping venture capital for biotechnology firms. The case of interferon reveals the complex institutional relationships involved in new technologies. It engaged scientists and politicians in marshalling resources to develop the technology; it attracted the interest of the press; and eventually it succeeded in mobilizing public curiosity and venture capital. The academic community, the government and the private sector developed close relationships.

The book points to the controversial policy issues raised by these relationships — the effect of extra-scientific factors such as media attention in setting research priorities, for example, and the influence of commercial interests on academic research and on the behaviour of the scientific community. It also deals with the conflicts over open disclosure of research findings, the manpower problems of a rapidly expanding scientific and technical field, the ethical and legal consequences of the changing relationship between science and industry, and the complexity of funding patterns that arise from the involvement of both the public and the private sectors.



"Forget the heroin business, Bugsy! I've something more profitable lined up!"

ACCORDING to a report prepared by the US brokerage and investment firm Bache Halsey Stuart Shields, interferon sales could have a world wide market of $2 billion a year within three to five years. The report, summarised in *European Chemical*

Interferon fever — cartoon and news report from *Nature* May 1980.

Panem is at her best when she discusses the history of the development of interferon, and its scientific importance in molecular biology and immunology despite its failure in the short term as a therapeutic agent. She writes skilfully of the dynamics of the biotechnology industry and the role of media, both in shaping research priorities and attracting industrial interest. The people, the firms, are real, and their activities are richly portrayed. However, she presents the key policy issues as little more than a list. There is much more to be explored in the problems of secrecy so often observed as a consequence of the new associations between science and industry engendered by the growth of biotechnology. How great a problem is secrecy in science? What kind of contracts are being developed, and what is their effect? What is the actual influence of non-disclosure on scientific productivity?

One suspects the changing relationship between science and scientists and their sponsors is also changing the prestige system in science. Rewards are increasingly coming from consulting contracts, from participation on corporate boards. What does all this mean? At some universities entire biology departments have been redirected to research in genetic engineering and biotechnology-related research. What does this imply for the training of students, and more generally, for the wider range of biological research? There are also many questions to be asked about the selling — the overselling — of science, and Panem makes a fascinating observation about the role of "hype" on funding patterns. Between 1978 and 1981 the increase in government spending on interferon research greatly outpaced the overall rate of growth in biomedical research in general. Yet the peer review ratings were much lower, suggesting how politicians more than peers can shape scientific priorities. What are the implications of this pattern of funding "hot" research subjects before good proposals are developed? When commercial interests influence research funding, what does this do to the ideal of science as an autonomous, neutral activity? While the focus on fashionable areas of research has implications for science itself, there are perhaps more serious implications for the rest of us.

The overselling of interferon also bears on the credibility of science as a whole. In many cases, scientists contributed to media publicity, believing that it was an effective way to attract support. But this also created inflated public expectation and then disillusion as delivery of therapeutic benefits was delayed. As one journalist cynically observed about interferon, "At only a hundred trillion dollars a gram, this miracle drug has a future".

Panem competently outlines the policy dilemmas intrinsic to this revolutionary area of research, but she offers no point of view. Her descriptions of the "interferon crusade" are balanced, but bland: she takes no position on the many controversial issues that she lists. Her view, as stated in the introduction, is that "understanding the interferon experience may prove useful in predicting future problems of biomedical research [and] other scientific areas as they experience technical revolutions". She has laid the groundwork for such an understanding, but it remains for others to examine with a more critical and analytical eye the dilemmas that are outlined in her book. □

Dorothy Nelkin is a Professor in the Program on Science, Technology and Society, and in the Department of Sociology at Cornell University.

Renewed interaction

Peter D. Moore

Allelopathy, 2nd Edn.
By Elroy L. Rice.
Academic: 1984. Pp.422. $55, £42.50.

PERHAPS the best possible excuse for writing a second edition of a book is when the author changes his mind about the definition of the title; this becomes even more imperative when the title is a single word, such as allelopathy. When Rice wrote the first edition of this book in 1974, he adopted a conservative definition and confined his attentions to those chemical interactions between plants (including microbes) which result from the release of compounds into the environment and which cause harm to the recipient. He now feels that the definition was too narrow and that the term should include stimulatory interactions, and in this respect he comes into line not only with the coiner of the word (Molisch) but also with many others who use it.

Predictably, wider use of the term has resulted in the second edition including information which was excluded from the first. Hence it is a fatter book. But there is also a marked change in emphasis, for almost a third of this edition is devoted to what may be regarded as applied aspects of the subject, such as the importance of allelopathic studies in agriculture, horticulture and forestry. Further semantic problems occur even in these fields, as in the case of such well-known weeds as *Camelina sativa* in flax fields. It has long been known that flax plants in the immediate vicinity of the weed suffer reduction in growth rate, but is this due to allelopathy or to "competition" for some environmental resource such as nutrient ions? Both views have been held by various workers and chemical tests on plant exudates have not clarified matters, for leaf washings of *Camelina* have often been found to stimulate the growth of young flax plants. The latest work quoted by Rice suggests that benzylamine, secreted by *Camelina*, stimulates flax at concentrations below 100 ppm, while above 200 ppm it serves to inhibit it. So here we encounter problems both in the definition of allelopathy and of competition which are currently dogging the study of this subject.

In the analysis of allelopathic interactions in natural vegetation, emphasis has often been placed upon the occurrence of bare patches around plants or of patterns exhibited by plant populations. This work is thoroughly reviewed here, both for herbaceous and for woody species. The involvement of microbial elements in the plant interactions also receives due attention, as for example in the discussion of nitrification and the extent to which late successional species produce chemicals which inhibit the process and thereby gain advantage over those species with the potential to replace them. This is a subject which generates strong feelings among ecologists and it is well reviewed by Rice, who, predictably, comes down in favour of the idea of succession suppression by dominant plants.

From the ecological, we are taken into the realms of physiological and biochemical studies of allelopathy, including the chemical nature and the mode of transfer and action of the active agents.

The subject of allelopathy is one which transgresses a number of disciplinary boundaries and which can be expected to interest a wide readership. The first edition of this book certainly spent more time off my shelves than on them and is now very well worn. Such intensity of use, which I am sure is widespread, coupled with the amount of research into allelopathy over the past ten years, amply justifies the publication of a second edition even if the author had not revised his opinion on the definition of the term. □

Peter D. Moore is Reader in Ecology in the Department of Plant Sciences, King's College, University of London.

Ice age development

G. de Q. Robin

Ice Sheets and Climate.
By J. Oerlemans and C.J. van der Veen.
Reidel: 1984. Pp.217. Dfl.90, $34.50.

Ice Sheet and Climate Modelling would have been a better title for this work, which aims to help graduate students bridge a gap between glaciology and climatology. The authors' views and research contributions are presented concisely in relation to brief outlines of our knowledge of the climate system and of the work of other modellers.

In 12 short chapters descriptions and relevant equations are presented, starting with the global climate system. Here the energy balance, transport by atmosphere and oceans, and related effects of albedo feedback on these systems are covered. A largely mathematical outline is given of ice dynamics, including flow of ice shelves and grounding lines problems. Economical methods of numerical modelling are then introduced, and are later applied to problems of growth, decay and stability of ice sheets in the Northern Hemisphere and the climatic stability of ice sheets of Greenland and Antarctica.

Adequate modelling of vast ice sheets must involve their heat budget and bedrock adjustment to ice loading. Oerlemans and van der Veen avoid lengthy analysis of frictional heat production from stress–strain rate distribution by equating the loss of potential energy to total frictional heat production in steady-state ice sheets. Their analysis of bedrock adjustment is similar to that found earlier works, but leads to more sweeping conclusions.

In the book, the approximate 100,000-year cycle of growth and decay of Pleistocene ice sheets is attributed to flow dynamics of the asthenosphere and ice sheet once growth is initiated. The authors place less emphasis than others on latitudinal variations of radiation with time as a controlling factor. Even so they consider such variations are one likely cause of initiation of ice ages, with the next suitable minimum at 65°N at 50,000 years being much more likely to start an ice age than the earlier but less severe minimum around 20,000 years. In the near future, they calculate, warming of the atmosphere by doubling of the carbon dioxide content will deposit more ice on Antarctica (where melting is negligible) while on Greenland total melting would increase more than accumulation. The assumption that ice flow changes are negligible over a short period leads to estimated mass changes of the two ice sheets that together would lower sea level by 0.1m in 250 years.

When linking heat flow to large-scale ice dynamics, Oerlemans and van der Veen reach similar conclusions to other studies but with two exceptions. They reject internal strain heating of cold ice and frictional enhancement of sliding by ice melting as likely causes of major ice surges, pointing out that some conditions that lead to surging of valley glaciers do not apply to ice sheets. They do however conclude that basal trapping of water beneath ice sheets could result in large-scale cyclic surging. At this point, in moving on to develop models that could work rather than first discussing if these are plausible, it seems that the cart is put before the horse. Surely beneath fast-moving ice streams, sometimes termed "continuously surging glaciers", water trapping must be stable and continuous rather than intermittent. Since such ice streams discharge a large fraction of Antarctic ice, the authors could query whether really large-scale surges are possible. Neither modelling studies nor field data seem to provide strong evidence in their favour.

Although it is apparent that English is not the authors' mother tongue, the book is clearly written and presented. Its broad conclusion is that ice-asthenosphere dynamics drive an ice age — once it is triggered off — rather than climatic factors driving ice sheet dynamics. Oerlemans and van der Veen do not claim to provide final answers, but they have produced a stimulating book that should, as they intend, result in further and more detailed research in this field. □

G. de Q. Robin is at the Scott Polar Research Institute, University of Cambridge.

● Cambridge University Press has issued paperback editions of *Theory and Experiment in Gravitational Physics* (by Clifford M Will) and *The Very Early Universe* (edited by G.W Gibbons, S.W Hawking and S.T.C. Siklos). Price of each book is £15, $24.95. For reviews see *Nature* **299**, 284 (1982) and **309**, 473 (1984).

Remains of humanity

Yoel Rak

The Origins of Modern Humans: A World Survey of the Fossil Evidence. Edited by Fred H. Smith and Frank Spencer.
Alan R. Liss: 1984. Pp.590. $70, £53.

IN THE late 1930s only the fact that a group of human fossil remains was discovered at the same site on Mount Carmel spared each specimen from being assigned to a different taxon — a "separate form of humanity". There can be no better manifestation of the changes in approach and thought regarding the appearance of anatomically modern *Homo sapiens* than the articles offered in *The Origins of Modern Humans*. There has long been a need for such a volume, to bring together data on the enlarged fossil record and demonstrate the innovations in our methods of assessment of that record. One is reminded that almost 30 years have passed since the appearance of the last attempt to summarize the state of the art in this area, *Hundert Jahre Neanderthaler 1856-1956*, published in commemoration of the hundredth anniversary of the first Neanderthal discovery.

Of the 11 contributions in the book, the first two are of a general nature. F. Clark Howell introduces matters with a comprehensive survey that in essence summarizes the contents of the whole volume. And as historical background and past ideologies must be considered in any attempt to assess theories of the appearance of modern humans, the second chapter, written by F. Spencer, concentrates on the history of the fossil discoveries and their interpretations. It is unfortunate, however, that the three main evolutionary models of early *H. sapiens* so clearly depicted by Spencer are again independently described several times in following chapters, creating a rather tedious redundancy.

The heart of the book consists of the nine remaining contributions, which are devoted to the skeletal remains found in various regions of the Old World and the New: Europe (covered by C.B. Stringer, J.J. Hublin, B. Vandermeersch; F. Smith; and D. Frayer); western Asia (E. Trinkaus); Africa (P. Rightmire, G. Brauer); east Asia and Australasia (M.H. Wolpoff, Wu X.Z. and A.G. Thorne); China (C.L. Brace, Shao X-q. and Zhang Z-b); and the Americas (R.C. Owen). The articles are to a large extent organized according to a similar pattern. Initially there is an up-to-date account of the fossil record and its chronology, thus allowing the reader to become acquainted with a number of little-known specimens, that being followed by interpretation of the fossils, primarily phylogenetic. The geographical scope of the book as a whole is both broad and well-balanced; as such it is refreshingly free of the Eurocentric bias that, as the editors remind us, formerly plagued anthropological thought. Similarly, the range of approaches and opinions of the various authors reflects most of the current thinking on the topic. Only by close attention to the differences in approach to morphology and to the differing biological models of each author will the reader comprehend how the same group of specimens is considered "archaic", "primitive" and "generalized" by some, and "advanced", "specialized", "derived" and "modern" by others.

The views of most of the contributors are already well known, but publication of their current research in one volume provides for the first time an overall perspective on the appearance of anatomically modern *H. sapiens*. Because of the range and diversity of the contributions, the references accumulated here by themselves constitute a veritable treasure trove.

Still, there are some criticisms. Detection of the polarity of a morphocline in the period under discussion is admittedly difficult because of the relatively short time span involved, the polymorphic nature of the species, and, lastly, the fully modern volume of the brain in the hominids considered (a criterion which had dictated polarity in earlier stages of human evolution). Nevertheless, more of the contributors could have attempted to deal with the question of evolutionary trends through a more rigorous functional interpretation of particular morphologies. Culture with its effects on morphology is the primary topic of those who do offer an adaptive interpretation. Such an approach, along with precise dating, might ensure that a greater degree of certainty could have been accorded the somewhat speculative statements on migration, local development and replacement.

The importance and overall quality of the book are not seriously marred by several other deficiencies. A world map showing all the sites mentioned would have been most useful (the two maps which do indicate sites unfortunately portray the same region and most of the same fossil locations), while the quality of some of the photographs and drawings leaves much to be desired. A greater effort could have been made to standardize the names of fossils and sites; the skull from Saldanha, for example, appears under three different names. Finally, although the book deals with the fossil evidence, some reference to genetic studies would have been in order.

The editors and contributors to this book have provided us with an enlightening review of the problems surrounding the origins of anatomically modern *H. sapiens*. Readers will need some prior familiarity with the fossil record, and with anatomy and population genetics. For such people this will be an invaluable source of reference. □

Yoel Rak is a Lecturer in the Department of Anatomy and Anthropology at Tel Aviv University, Israel

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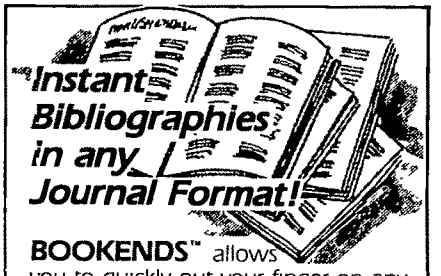
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Ultrastructuralism

Michael Ashburner

Insect Ultrastructure. Vol.1 The Ultrastructure of Gametes. Vol. 2 The Ultrastructure of Developing Cells.
Edited by Robert C. King and Hiromu Akai.
Plenum: 1982/1984. Vol. 1 pp.624, $55, £52.25. Vol. 2 pp.650, $85, £80.75. Two-volume set $127.50, £121.13.

IN September 1969 the Royal Entomological Society of London held a symposium called "Insect Ultrastructure". Of the eleven people who contributed to it five were, or had been, members of the Department of Zoology in Cambridge. Surprising, considering that the meeting was organized by an Oxford man. The present two volumes have 49 contributors, only one of them from Cambridge Zoology. But then only one, David Spencer Smith (to distinguish him from all the other eminent biologists, let alone Oxford professors, called David Smith), has contributed to both of these volumes and to the 1969 symposium. David Spencer Smith is, now, in Oxford.

Who will read such volumes as these, 31 chapters on the ultrastructure of insects? I suppose there are those who will read anything on structure if it is ultra, whether from bees or bats. There are, I know, those who will read (almost) anything on insects, from ultrastructure to sociobiology. For these readers the two books suffer from a serious lack of balance. Some of the chapters are very narrow, others paint on a broader canvas. For example at one extreme we have Sorsa's review of the ultrastructure of the polytene chromosomes of *Drosophila melanogaster*. I have no quarrel with Sorsa's chapter as a contribution to science (at least no quarrel worth airing in these pages) but the ultrastructuralist or entomologist reading these volumes should be warned that it is very much a review of Sorsa's own work and makes no attempt to review the field comprehensively. Moreover there can be few who require 10 pages of a detailed map of the banding pattern of chromosome arm 2L. An $85 book is not a suitable medium for its publication.

Another example of a review whose field is narrow is that by Rasmussen and Holm on the ultrastructure of meiotic prophase in the silkmoth, *Bombyx mori*. I am sure that this is a subject worthy of review. That is not my complaint. My complaint is that in a large and expensive work called *Insect Ultrastructure* the purchaser might expect a more comprehensive review of, for example, meiotic prophase in insects or, perhaps, of meiosis in *B. mori*. I must not give the impression that all of the chapters are as narrow in their compass as these. Many, indeed, will be of interest to biologists with a wide range of interests, David Spencer Smith's review of the ultrastructure of insect muscle being one such.

In addition to the two classes of omnivorous reader there are those (and here I include myself) who approach volumes like these with the attitude of a selective browser. We will read chapters in this book for one (or more) of four reasons: (i) because we need to know what is going on in a particular field of research (which may be our own); (ii) because we always wanted to know about, for example, neuromuscular junctions in the Orthoptera but never, before, knew who to ask; (iii) because we are intrigued by the title of a particular chapter; or (iv) because we once met Professor Kafatos at a conference banquet in East Idaho and always wondered what he did for a living. For the selective browser balance is irrelevant; these volumes are a lucky dip to which he can return over and over again.

It is interesting, for an outsider whose practical experience of insect ultrastructure is limited to peering over Nancy Lane's shoulder at a glowing screen, to compare Neville's 1969 volume and David (Spencer) Smith's 1968 book *Insect Cells: Their Structure and Function* with these volumes. Two overall impressions. The first is the importance of technical advances: not only that the slow but steady improvement in preparative techniques has led to far better electron micrographs, but of methods such as scanning electron microscopy (in its infancy in 1968), freeze fracture, chromosome spreading and reconstruction from serial electron micrographs that are now routine tools. The greatest contrast, though, is that the two earlier books, Smith's especially, left one with a very static view of structure, making the necessary relationship between structure and function difficult to draw. Now, however, I get a real feeling for dynamic processes, of the importance of the change in structure, and hence function, with time. It is impressive to see how much the study of insect ultrastructure is now the adjunct of the experimental biologist, rather than the preserve of the morphologist. □

Michael Ashburner is Reader in the Department of Genetics, University of Cambridge.

• Next week's issue of *Nature* includes the Spring Books Supplement, 25 pages of reviews from, among others, W.F. Bynum, Richard C. Lewontin, Nevill Mott, David Park, Owen Gingerich, David Bohm and Anthony Clare.

Sea slugs: beauty in biology

Elizabeth B. Andrews

Biology of Opisthobranch Molluscs, Vol. II.
By T.E. Thompson and G.H. Brown.
The Ray Society, British Museum (Natural History), London: 1984. Pp.229. £39.

THE delicacy of shape and the beauty of colouring of sea slugs, the animals with which this volume deals, have captured the attention and admiration of many zoologists, amateur and professional. Their agility and grace of movement contrast markedly with the drabness and ponderous crawling of their terrestrial relatives and with their own dreadful deadness when pickled. They have remained a rather neglected group by comparison with both prosobranch and pulmonate gastropods, however, due partly to their unreliable occurrence and partly to the fact that many species are particularly abundant in those marine habitats that are most difficult to explore — too low for shore collecting, too shallow for adequate boat-work. The first difficulty still remains, and some species are genuinely rare. Scuba diving has largely abolished the second, however, and one reason for the success of this book is that both authors are accomplished divers and have thus found not only more specimens for study but also more species new to the area covered by their work.

The authors offer a taxonomic survey of the nudibranch gastropods found in British waters, with keys and illustrations to help identification. They give brief definitions of subordinal and familial characters, and for each species provide details of appearance, anatomy, feeding, breeding and a brief synonymy. Local distribution is recorded in a series of maps, and foreign distribution is mentioned, and there are short discussions of taxonomic problems, a very complete list of references and a helpful index. The book also benefits from the provision of an "Epilogue": a summary of ideas and information relating to opisthobranchs published since 1976 when Vol. 1 appeared.

Illustrations are important in any book dealing with such photogenic subjects as nudibranchs, and comparison with The Ray Society's previous publication on the group, Alder and Hancock's *A Monograph of the British Nudibranchiate Mollusca* (1845–1855), with its beautiful figures, is inevitable. Thompson and Brown's work stands up to the comparison with distinction: all but two species are illustrated in a series of plates on which paintings of the animals stand against a black background, lifelike and attractive. In addition, each species is shown in black and white, a helpful guide for the student.

For all interested in this group of animals the book will be an essential acquisition. It is an excellent addition to the series which The Ray Society has produced for so long, and a worthy successor to Alder and Hancock's *Monograph*. □

Elizabeth B. Andrews is Senior Lecturer in Zoology at Bedford College, University of London.

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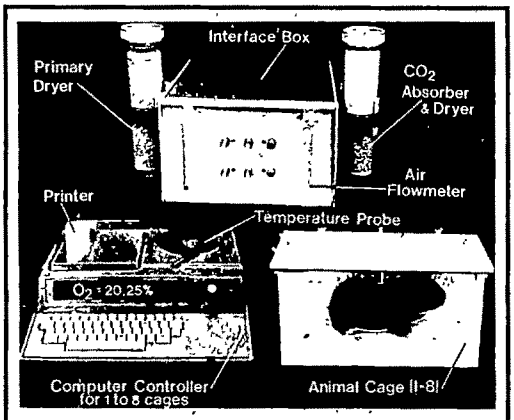
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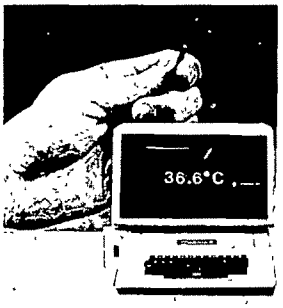
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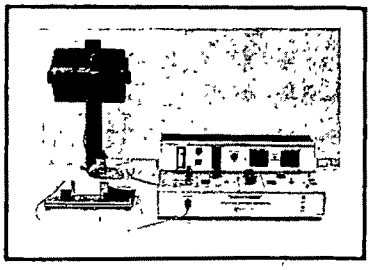
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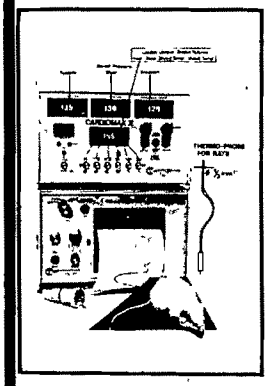
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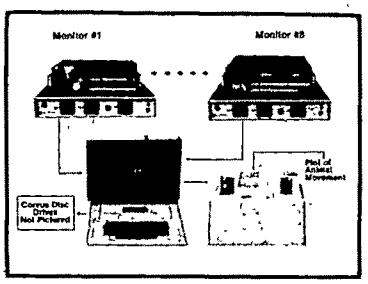


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Reader Service No.14

FASEB '85
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Booth 1429

New for FASEB at Anaheim

This year's meeting of the Federation of American Societies for Experimental Biology is at Anaheim, California, opening on 21 April.

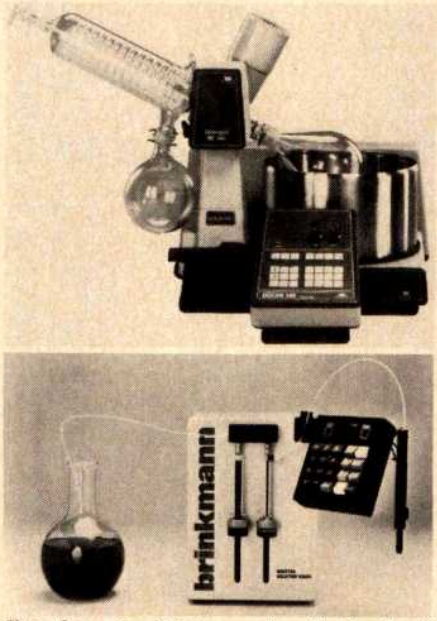
• Visitors to Vector Laboratories at Booth 2027 at FASEB can obtain the newest reference and applications list for the Vectastain ABC technique. Citing some 120 of the most recent publications using this ultrasensitive biotin/avidin-based immunoperoxidase system, this list is the third in a series. In addition to providing a compilation of current references, an index of specific applications is included, highlighting pertinent articles using lectins or monoclonal antibodies for immunohistochemistry, enzyme immunoassays, nitrocellulose blotting, DNA hybridizations and electron microscopy.
Reader Service No. 100.

• Two high performance gel filtration columns, Superose 6 HR 10/30 and Superose 12 HR 10/30, have been introduced by Pharmacia. Superose is a unique, cross-linked agarose matrix with high chemical stability over a *p*H range of 1–14. Prepacked in high resolution glass columns (1 × 30 cm), Superose provides high protein and enzymatic recoveries, reproducible separations and long column life. The two products cover a molecular weight range for globular proteins of molecular weights between 1,000 and 5 million.
Reader Service No. 101.

• At the 1985 FASEB show, Bio-Rad Laboratories (Booth 2220) will feature the new Protean II vertical electrophoresis system, a new chromatography system for preparative scale monoclonal antibody purification, and new protein microanalyser. In addition, a variety of chromatography and electrophoresis products of special interest to life scientists are on view. These include: HPHT-MAPS monoclonal antibody purification system for removal of protease, transferrin, albumin, and other contaminating proteins from up to 20 mg of mouse, human, and rat IgG and IgM. The Quick-Check analyser for purification monitoring or protein profiling in 15 min. A protein chromatography system with six complementary chromatographic mechanisms to produce highly purified, active proteins. A data acquisition system with software reintegration, peak naming, and data storage. A fixed-wavelength UV monitor for high sensitivity detection at 280, 254, and 214 nm. A new video densitometer allowing data to be viewed, manipulated, and optimized directly on the video screen. And Immun-Blot, a range of non-isotopic picogram-sensitive blot detection kits.
Reader Service No. 102.

These notes are based on information provided by the manufacturers. Use the reader service card bound inside the journal to obtain further information.

• A new ion-exchange medium that can be packed more quickly and easily than conventional gels, and allows faster flow rates for high-speed protein isolations, is being introduced by Waters Chromatography Division of Millipore Corporation at the FASEB/ASBC meeting. On view on Booths 2633–2641 and 2732–2740, Waters Accell ion-exchange medium is a silica-based chromatography packing featuring a patented copolymerization process that exploits the properties of a rigid separation medium while giving excellent recovery of biological activity. The patented copolymerization process encapsulates the rigid wide-pore silica base to produce low, non-specific binding. The rigid structure permits linear scale-up at all flow rates and pressures, from analytical to preparative to process chromatography, using the same methods and chemistry.
Reader Service No. 103.



Two from the Brinkmann booth, the Buchi rotary evaporator (top) and Brinkmann's digital diluter.

• At Booths 2500–2505, Brinkmann Instruments Co. is showing the new Buchi medium pressure liquid chromatograph for preparative-scale isolations and Brinkmann homogenizers for use in the industrial or biomedical laboratory. Brinkmann is also introducing the new Buchi rotary evaporator Model RE-140, which provides full automatic evaporation via a microprocessor-controlled keyboard. Other products include the Brinkmann digital diluter 9200, featuring continuous selection of volumes from 1 μl to 10 ml and fractional volumes to 0.1 μl.
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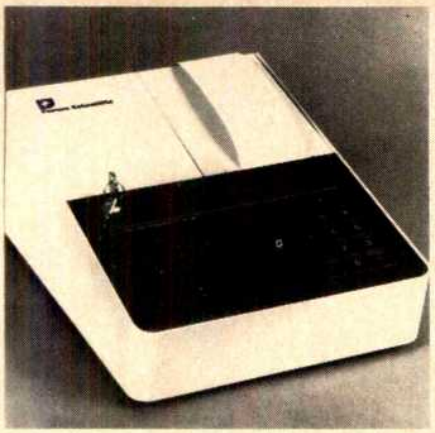
• Pierce Chemical's Diphenyl-8 GC capillary column conditioner is specially purified diphenyltertramethyl-disilazane (DPTMDS). With the higher temperatures and increased sensitivities common to capillary column work, the presence of silanol groups on the stationary phase and column can cause major chromatographic problems. Diphenyl-8 eliminates the symptoms characteristic of residual silanol, prolonging the lifetime of a column by reducing stationary bleed. It deactivates silanol groups on the solid support and acts as a "cleaning agent" for column residues capable of being derivatized.
Reader Service No. 105.



The new Orion cyanide standard.

• Orion Research Incorporated has introduced convenient packs of cyanide standard for use with cyanide electrodes as a calibrating solution. Each reagent pack contains 10 sealed glass ampules, with 50 ml of 100 p.p.m. cyanide standard in each ampule. The standard can be used directly as a 100 p.p.m. calibrating solution or diluted to provide additional dilutions.
Reader Service No. 106.

• The J.T. Baker Chemicals range for lipid screening has been expanded by the addition of two new reagents for cholesterol and triglycerides monitoring. These reagents are formulated according to the CHOD-PAP and GPO-PAP principles respectively. The cholesterol reagent is in a liquid, ready-to-use configuration. The reagent remains stable for up to 12 months and shows linearity up to 20 mmol l^{-1}. The triglycerides reagent is lyophilized and is stable on reconstitution for 3 weeks at 2–8°C. Application sheets for lipid screening reagents are available for a variety of clinical chemistry analysers.
Reader Service No. 107.



● Forma Scientific's new desk-top programmable digital recorder/alarm monitor, the Model 1525, can receive input from, and record any combination of, eight conditioned millivolt signals, such as temperature, per cent CO_2 and O_2, per cent RH, and *p*H. The sensitouch keyboard makes it easy for the user to program the high/low deviation limits for any specified condition. The ink printer records date, time, function, and input number, plus a printout at programmed intervals. Additional printouts are furnished along with audible and visual alarms in the event of a deviation beyond setpoint limits.
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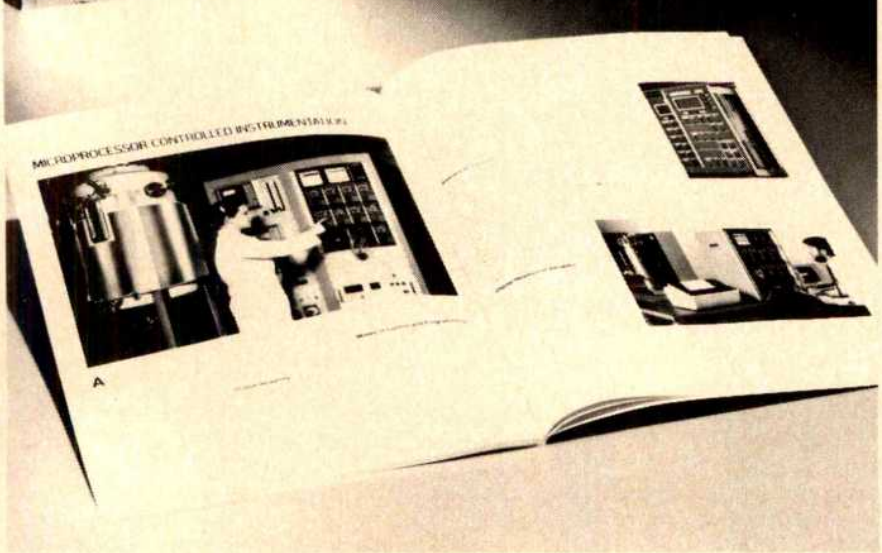
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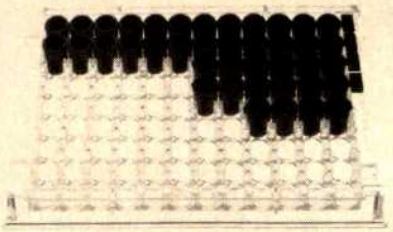
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Removawell, Dynatech's new plate holder.

● Dynatech Laboratories has added a new Removawell plate holder for Microtiter and Microelisa plates. The plastic disposable Removawell system allows the user to build up an 8 × 12 well plate from strips of wells or from individual wells. Up to 96 wells in total can be held firmly in any configuration. The Removawell holder is designed so that it may be inverted with complete safety when emptying the contents of the wells, regardless of the number of strips or wells present. When assembled with wells, the whole Removawell system has the rigidity and strength of a conventional Microtiter plate, and it may be stacked for easy storage.
Reader Service No. 109.



A splash-proof touch-sensitive keypad controls the WPI pH meters.

● The 700 Series microelectrode amplifiers from World Precision Instruments are designed principally for intracellular recording using fluid filled, high impedance glass microelectrodes. All units feature ultra-stable, gold-plated input probes, low noise, negative capacitance and electrode test capabilities. Applications include intracellular and extracellular potential measurements, impedance and ion selective measurements. Also new from WPI, the HI 8417 and HI8418 microprocessor-based *p*H meters are programmed for easy daily laboratory use. Both meters feature automatic recognition of three different buffers and automatic temperature compensation. Two large LCD displays allow for simultaneous viewing of *p*H (or mV) and temperature. The HI 8418 includes a printer and a numeric keypad. The printer can list not only the *p*H/mV readings, but also the time, date and calibration parameters. The numeric keypad can be used to enter user-defined buffers and intervals for timed printing of data.
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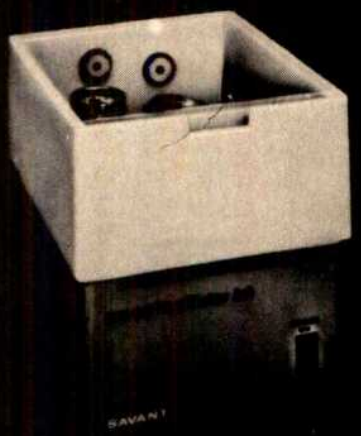
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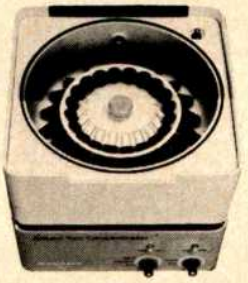
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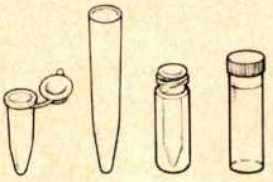
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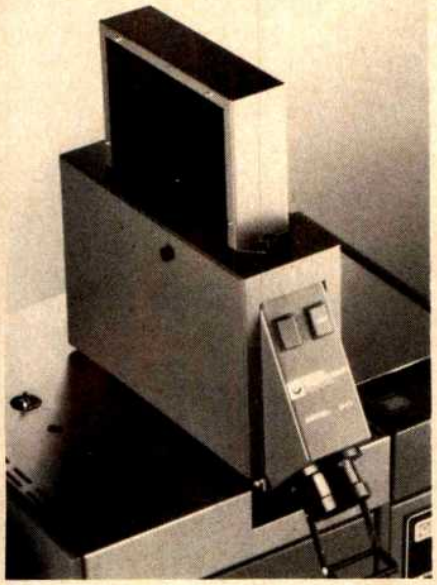
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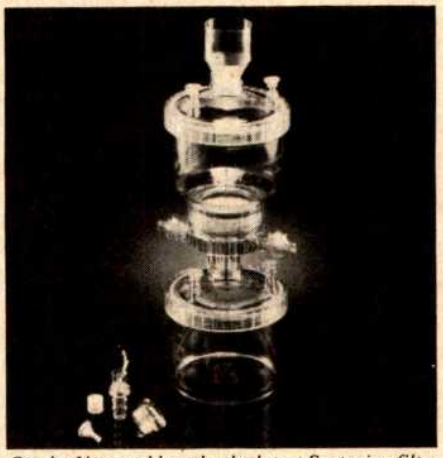


Robotics makes Packard's new sample injector simple to use, with both packed and capillary chromatography columns.

● Packard's "One-Shot Samplers" are injectors for gas chromatography that offer operational simplicity and security. The operator needs only to position a sample vial and press the "start" button to effect injection and begin the analysis. Because the sample volume injected is accurately and reproducibly measured, the use of the external standard (ESTD) method becomes much more attractive; in many cases it can replace the internal standard method (ISTD). Two models are available, the Model 941 can inject into either the front or rear injection port. The desired port is software selectable, this feature facilitates the automation of identification and duplication of analyses. Two different analyses can therefore be run. The Model 940 is similar but allows injection only into the injection port above which it is mounted. Both models are suitable for packed and capillary columns.
Reader Service No. 111.

● New England Biolabs have developed an *in vitro* method for creating novel DNA cleavage specificities. This simple procedure involves the methylation of the DNA with a site-specific methylase followed by cleavage of the DNA with a restriction endonuclease whose recognition sequence overlaps that of the methylase. Only a specific subset of the restriction endonuclease sites will be cleaved (see *Nucleic Acids Res.* **5**, 5165-5173; *Biotechniques* **2**, 218-222). The latest additions to New England Biolabs' list of over 90 restriction endonucleases include the following: *Bsm*I (GAATGCN/); *Fsp*I (TGC/GCA) (= *Mst*I); *Nhe*I (G/CTAGC); *Ssp*I (AAT/ATT); *Spe*I (A/CTAGT); and *Sty*I (C/C(A,T) (A,T)GG). New DNA methylases include; M.*Hph*I (TCACC); M.*BamH*I (GGATCC); M.*Hae*III (GGCC); and 'dam' (GATC). New M13 cloning vectors from NEB are: M13mp18 RF I DNA and M13mp19 RF I DNA.
Reader Service No. 112.

● The new polymer-based columns from Hamilton Co. of Reno, New York, are intended to extend the limits of HPLC and ion chromatography. Hamilton polymeric reversed-phase (PRP) resins accept eluents between *p*H 1 and 13, whereas silica tolerates only *p*H 2 to 8. For HPLC, PRP-1 columns separate virtually all classes of compounds, with no need for ion-pairing reagents. Because PRP particles are chemically identical from surface to centre, selectivity variations common to silica cannot occur. Columns are available with different particle sizes (5 and 10 micrometres) and in a variety of lengths (15, 25 and 30.5 cm) to meet most chromatographic needs. Low-cost bulk packing is available for preparative-scale separations. For ion chromatography, the PRP-X100 achieves rapid separation of the common anions. Unlike silica, the *p*H tolerance of PRP-based resins permits high-resolution separations of cyanide, sulphide, silicate, borate, and other anions requiring high-*p*H mobile phases. The 10-micrometre PRP-X100 columns are compatible with existing HPLC and ion chromatography equipment, and are available in 10, 15, and 25 cm lengths.
Reader Service No. 113.



On the Vangard booth, the latest Sartorius filter holder.

● A multipurpose filter holder, manufactured by Sartorius GmbH, is now available in the United States from Vangard International. Applicable for vacuum or pressure filtration, the multipurpose filter holder is integrally moulded of clear polycarbonate for strength and to ensure repeated autoclavability at 121°C steam. Applications for the filter holder include clarification and/or sterilization of liquids, and microbial analysis such as sterility testing.
Reader Service No. 114.

● A new brochure available from Tekmar describes the company's line of high shear dispensers/homogenizers. Units are available in sizes from laboratory to full-size production. Standard machines are available for batch and in-line processing of liquid/liquid, solid/liquid and gas/liquid fluids. Special metal alloys and standard unit designs are also available.
Reader Service No. 115.

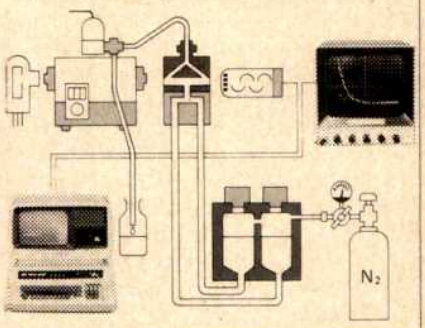
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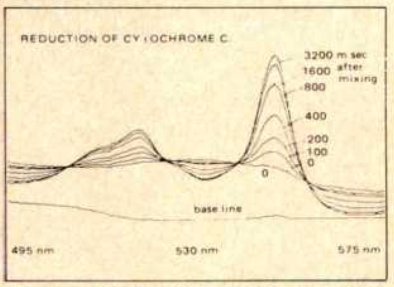
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● The K531 Dako PAPA kit System 20 is designed as a research tool for the demonstration of myelin basic protein (MBP) using the peroxidase anti-peroxidase staining technique. The primary antibody in this kit represents a pool of optimal antisera selected from many immunized rabbits. This polyclonal antibody shows very good reactivity with the antigen even under the adverse conditions encountered after tissue is fixed in formalin. In addition to 7 ml of primary antibody, a comprehensive set of reagents is provided in working dilutions. Five control slides for the initial use of this kit are also included. K531 has a minimum capability of twenty tests. This routine system comes with simple instructions for those with limited or no previous experience with the immunoperoxidase technique.
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Reader Service No.163



Cherry, pine and clove scents overcome less attractive odours—Odo-Clave from Bel-Art.

● The unpleasant odours emitted during autoclaving can be countered using Bel-Art Products' Odo-Clave deodorant disks. Two new scents have been introduced, pine and cherry. Each 1½-inch disk is impregnated with a special heat released deodorant specifically designed to be activated when exposed to the high temperatures of the autoclave. The pine and cherry scents, like the original clove scent, all serve to neutralize, not mask unpleasant odours.
Reader Service No. 117.

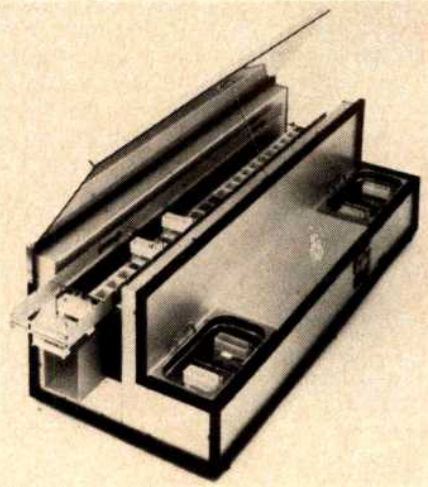


The Cavro sample processor and diluter gain flexibility with a new hand-held probe.

● Cavro Scientific Instruments, Inc. has introduced a disposable-tip hand probe for use with their automated IQ 190 sample processor and Model 1500 pipettor/dilutor. The hand probe ensures zero cross-contamination and is ideally suited for applications using enzymes, radioisotopes or biohazardous materials. The hand probe can be used for liquid transfer, or can pipette and dilute in a single operation. Multiple dilutions (serial dilutions) can also be performed using a single tip. Aspirations up to 75-microlitres can be performed using 200-microlitre disposable tips. Dilution ratios are easily controlled by adjusting the dispense volumes of the two syringe pumps on the instrument. The natural grip, weight and angle of the hand probe are optimized for ease of use and to minimize fatigue. Because the hand probe is used with an automated pipetter/diluter, variable results normally associated with extended use and operator-to-operator variations are eliminated.
Reader Service No. 118.

● A new applications note from Amicon, entitled "DNA Concentration and Desalting Data" covers the processing of dilute DNA solutions by means of simple centrifugal ultrafiltration with Amicon's disposable Centricon microconcentrators. Concentration and desalting of plasmid DNA and plasmid restriction fragments is reviewed. Ask for Publication No. 532.
Reader Service No. 119.

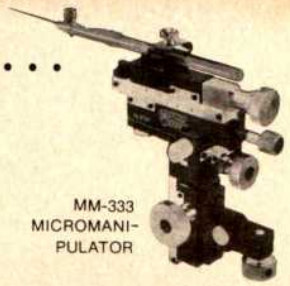
● Streptavidin and biotin are being used increasingly as bridging reagents for detection of proteins, cells and nucleic acids, as well as isolation of cells and proteins. Streptavidin exhibits the same high biotin-binding capability as does egg white avidin, but with much less nonspecific binding. The result is high specificity and sensitivity with very low levels of background. Bethesda Research Laboratories has compiled a comprehensive illustrated guide, "Streptavidin: Applications in Detection of Proteins, Cells and DNA". This free 12-page booklet begins with an explanation of the powerful and versatile streptavidin/biotin interaction. The guide details many applications of streptavidin/biotin methodology, including: detection of proteins by immunoassay and immunoblotting techniques; detection of cells by light, fluorescence and electron microscopy; nonradioactive detection of DNA at 2-5 picogram levels; isolation of cells by fluorescence activated cell sorter; and isolation of proteins by immobilized streptavidin.
Reader Service No. 120.



The Hacker slide-stainer.

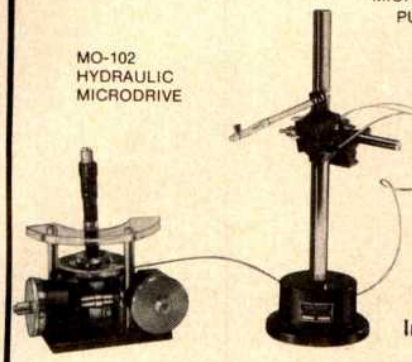
● For histology and cytology, the Hacker Instruments continuous slide stainer features linear operation through 18 stations. Immersion time is variable between 6 and 12 min, with a drain time of 3-60 s. The holders transport 22 slides each through the stations, and up to 5 holders store before and after processing. Holders may be agitated and stations 2-17 are plumbed for running water. Throughput is up to 600 slides per hour at a dwell time of 2 min per bath. All conventional and special stains and rinses can be used to give uniform standardized slides.
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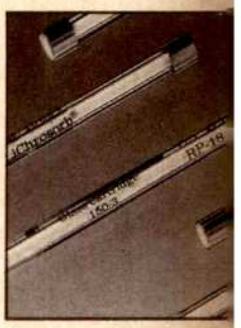
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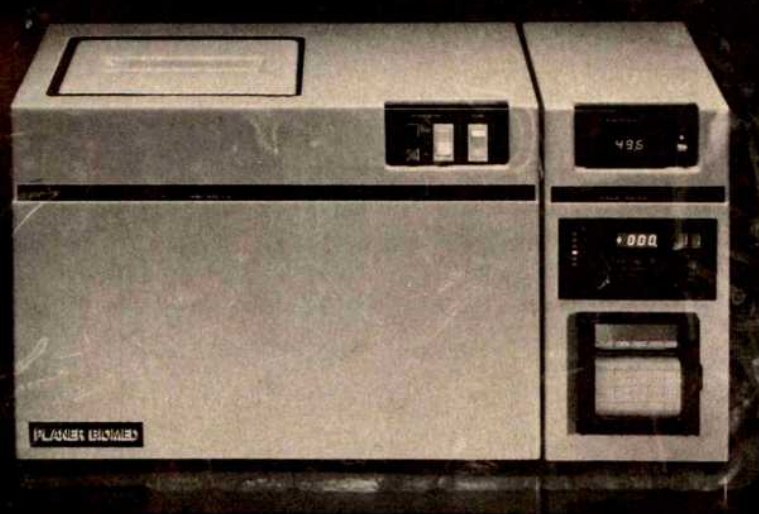
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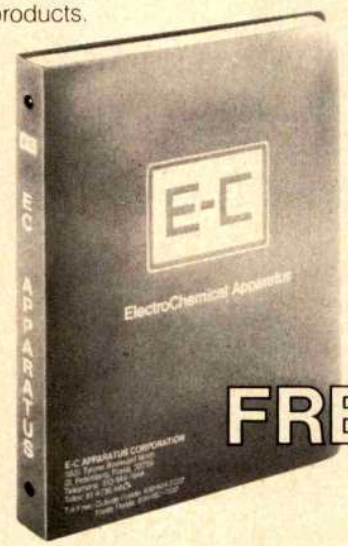
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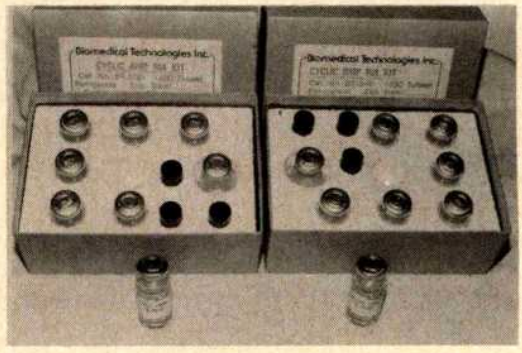
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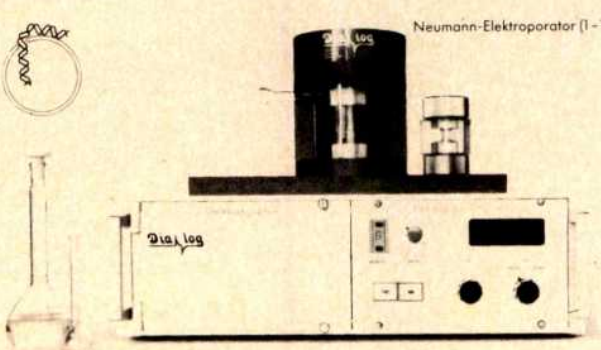
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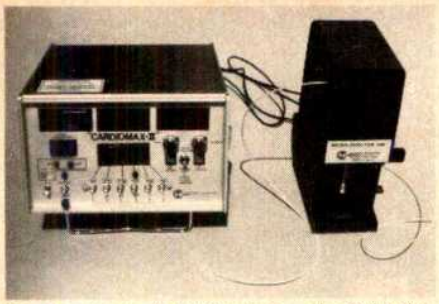
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nature

NATURE VOL 314 25 APRIL 1985

Are the philistines in charge?

A curious argument in the London Times *suggests that those who would make the British universities more productive do not understand what science is about.*

SIR Douglas Hague, the chairman of the British research council charged with supporting social science, has been a valuable influence among the grander councils of the research establishment in the past two years. He has rightly raised awkward questions (still not answered) about the management of the British government's support for civil science, in particular asking whether the Advisory Board for the Research Councils is constituted in a way that will allow it to do the job intended for it. Sir Douglas is also a member of the committee looking into the future of British membership of the European centre for high-energy physics at Genèva, and is widely expected to be found among those who advocate pulling out when the report appears in a few weeks. Earlier this month, he argued (in *The Times*) the case for stronger links between industry and academic science, and in passing made a side-swipe at the academics of the University of Oxford for having denied the Prime Minister, Mrs Margaret Thatcher, an honorary degree, which is also fair (see *Nature* 7 February). If Sir Douglas Hague is a philistine, he is both measured and moderate in that role, more a gadfly than a real philistine.

The same cannot unfortunately be said for his supporters, especially Dr K.A. Harrap, who described himself (*The Times*, 19 April) as having been, until recently "head of marketing" at the Natural Environment Research Council. His premise is that British science, which is "fit, able and creative", has for too long been dependent on only the government for support. Dr Harrap continues by acknowledging that there has now been a change of circumstances, but that it will not suffice that the scientific community should simply cry "foul". So, he concludes, scientists must "publicly emphasise their wealth-generating capability" not only in the public interest but so as to "demonstrate to the public and its government that science is good value". For good measure, he asks that "publicly funded scientists themselves" must demonstrate that they can turn "intellectual strength" into wealth, and that if, in the process, the "cutting-edge of blue-skies research becomes somewhat blunted so be it". The meaning of the phrase is probably clear even if its imagery is a little confusing.

The reasons why this argument is false, and dangerously so, deserve to be widely understood, for there is every reason to suppose that the same mistaken conclusions account for much of the British government's indifference to the damage it has been doing to the scientific enterprise during the past five years. (Dr Harrap, for that reason, should not think he has been unfairly singled out for criticism; indeed, he might even claim credit for having made public, perhaps unwittingly, a line of argument that should be better known.) The issue is the balance that should be struck between basic and applied research in a country such as Britain which has, for far too long, supposed that the economic problems that have afflicted it do not exist, and the question of the role of basic research when wealth-creation, in Dr Harrap's language, is the goal.

No harm is done by conceding Dr Harrap's premise, and its antecedents. British research support has been unduly monolithic. British academics have been too uniformly inclined to measure their success in exclusively academic terms. It might be added that much of the reason why British academic research has been industrially disappointing comes from the unwillingness of government departments and industrial companies in Britain to credit British academics with being capable of generating valuable innovations. If it were otherwise, the defence and industry departments would be more than the minuscule sources of research support they are at present, while the handful of British universities that have been glad to welcome industrial support would have prospered to the envy of the others. It serves no purpose for anybody to lay Britain's economic poverty at the doors of the academics.

It is also possible to concede the continuation of the argument, that crying foul on a change of circumstances serves no purpose. The British academic community as a whole, not just the scientific part of it, was far too slow to recognize the threats to its integrity when these first began to appear more than a decade ago. Not surprisingly, the pretence that there was nothing wrong has now been overtaken by a sense that everything has turned to dust and ashes, and there is nothing to be done. In its own interests, the academic community in Britain should accept the Hague/Harrap argument that the academic community should recognize the serious character of the government's economic problems, show willing in helping solve them and be more imaginative in exerting itself in its own interests.

So how, when so much is conceded, can the Harrap line be mistaken? Because it makes the false assumption that prescriptions that can fairly be held to apply to the scientific community should apply to all scientists individually. And because it goes on to conclude that it would be possible for a country such as Britain, seeking to make its way in a more competitive world by harnessing all "publicly funded" researchers to the same goal of wealth creation, to abandon imaginative research with no foreseeable application without quickly running into trouble. Both conclusions are false. Present circumstances probably require (and will certainly drive) more academics to look for industrial projects on which to work, but why should the same recipe apply to each and all of them? And the second, that imaginative research is "for the time being" dispensable, is untenable, at least so long as it is intended that the education of students should be more than mere paper and that the applied research of future decades has some grist to feed its mill.

This is where the philistines go wrong. They forget, or they choose to overlook, that the scientific enterprise is all of a piece, and that while the balance between the different parts of it can be changed, the belief that some parts of it can be dispensed with altogether is an error born chiefly of ignorance. But what about Japan? Sir Douglas Hague's original protest raised this question but without conviction. It is, of course, a red herring, as the Japanese now seeking to legislate for more creativity within their system would be the first to confirm. Japan has done well, over the years, by also adapting technology from elsewhere to modern market needs, often the needs of markets elsewhere. But even Japan is not sure of sustaining its technological growth beyond the point reached by others with its own unembellished basic research. And while the Hagues of this world may understand the subtlety of the problem, they should choose their words more carefully so as not to give a licence to those who do not or prefer not to understand. In Britain just now, there are too many philistines of that kind roaming the streets and even the corridors of Whitehall. □

Dollar fragility

Why must researchers worry about the dollar? Because research funds may vanish.

LAST week's predicted further fall in the value of the US dollar is not the first this year, and will not be the last. Last month's tumble was attributed to the financial difficulties of several Ohio semi-banks and last week's to the estimate of slower US growth this year (1.8 per cent) than last (6.5 per cent), but these are occasions, not causes. The fragility of the dollar is a consequence of the US government's huge budget deficit and of the certainty that even if the administration and the Congress agree that it should be cut, the effects cannot appear until the beginning of the next financial year in October. Can the world wait that long?

The fear that time is running out is barely concealed. Even finance ministers at last week's meeting of the International Monetary Fund in Washington allowed their determination not to be seen to rock the boat to be compromised by anxiety. What worries them is that the US administration is now a tight box. Either it will have to cover its deficit by increasing interest rates still further or give up trying, print money instead and put up with the inflation that would follow. The explanation that last week's fall was triggered by people's calculations that the administration would now try to stimulate the economy by reducing interest rates is not to be taken seriously, for cost-free options have gone the way of the free lunch. Nor does it make sense to ask, as some do, that other industrialized nations should assume the burden of stimulating economic growth by government policy, for inflation-free growth outside the United States will be possible only when the flow of funds to the United States in the past few years has been substantially redirected, adding to the problems of the dollar.

This is why talk of monetary reform has become fashionable again. Even Mr James Baker, the new Secretary of the US Treasury, is in favour. The trouble is that nobody knows what should be done. Things have changed a lot since John Maynard Keynes went off to Bretton Woods in 1943 and drafted the monetary rules by which the world lived for the succeeding thirty years. The essence of Keynes's recipe was a blend of currency stability by means of declared exchange rates and a mechanism for the supply of credit, through the International Monetary Fund. One change since then is that there is now almost a surplus of international credit, much of it stimulated by the steep increase of the price of oil in the 1970s, whose movement is uncontrolled and hard to anticipate. Another is that the financial markets have become technically more efficient and sophisticated than Keynes could have imagined. The needs now are to find ways of giving currencies a measure of stability even when they are traded freely, and to shield developing countries from the damage they can suffer from these processes. After a decade in which most governments have been indifferent to these needs, it is gratifying that most governments now accept the need for reform.

Part of the trouble with the present system is that huge sums of money can slop about within it, in pursuit of the marginally different interest rates on offer at different places. The volatility of the system would be much reduced if only governments would agree to limit the proportion of their borrowing that consists of short-term debt. (In a typical week, the US Treasury now raises between $10,000 million and $20,000 million of short-term money, most of it to cover maturing debts and not the underlying deficit.) Self-denial like this by governments would not be very different from what governments require of their commercial banks. In the process of reform, Europe has a particular responsibility (and opportunity). A more thorough coordination of European arrangements for coordinating monetary policy would increase the inertia of the system without, in the long run, biasing the ways in which money moves. The European Monetary System, which ties together major European currencies (but not sterling), is a start but an imperfect one, while it will be decades before the European accounting unit (called the ECU) will become a currency. One objective, now, should be to set up a pan-European central bank with the funds and the independence to be effective.

The monetary problems of the developing countries are more intractable, partly because most governments do not accept the logic of the need to act. Why else would so many rich countries still restrict the import of simple manufactures, but not the raw materials on which many developing countries are too dependent for what passes for prosperity? While restraints like this persist nobody should be surprised that developing countries cannot easily attract the funds they need for development (although they are also, often, their own worst enemies by the political hazards with which they confront potential investors). In this sense, last week's meeting of the International Monetary Fund at Washington must know that its talk of insuring private companies' investments in developing countries, by some mechanism yet to be devised, is a palliative , not a solution. The monetary problems of the poor will be solved permanently only when the industrialized world accepts that they have a right to earn their way out of trouble, and is then prepared to help them do so through agencies such as the World Bank, designed for just this purpose. □

Tarnished initiative

The offer to allies of a share of the US defence budget has been indelicately handled.

THE second subject (monetary policy is the first) that will preoccupy the summit meeting of the major powers due next month is that of what should be done about the US plan to build a defence against ballistic missiles, sometimes known as the Strategic Defense Initiative, but which is otherwise star wars. European governments represented at the meeting will be in a fix. Sceptical of the scheme from the outset on the grounds that it would probably be ineffectual and that the development of such a system would cause more international trouble than even success would be worth, and in some cases fearful that success would lead to a US abandonment of Europe, they have been tempted against their better judgement into collaborating with the planned technical developments (see p.660). For many, the principle seems to be that they will then at least know what is going on.

The US government's position is not very different. There was nothing in President Reagan's speech in March 1983 to suggest that star wars was intended to be collaborative venture, or even that the governments of Western Europe could fail to share the president's own sense of wonder at the supposed benefits of a world (or part of it) blessed with immunity from nuclear attack. In the event, the scepticism of European governments blended with the administration's own sense that there may be safety in numbers in the trying months ahead have combined to persuade the United States that collaboration would be worthwhile. The reluctance, or surprise with which the administration has come to this view is clear from the letter from Mr Caspar Weinberger to the political heads of potentially collaborating countries last month: in these days of word processor, it is surely needlessly offensive to send what seems to be a circular letter, in which recipients are invited to render "your nation's" response.

As things stand, there is no way telling what European states should say. The offer of collaboration is too innocent of detail to be appraised. The governments that fear that collaboration will be a formal matter only may be right. Those who hope to make money out of the scheme may, alternatively, hit the jackpot. But nobody can know for sure — and certainly not within the 60 days first specified in Mr Weinberger's letter. But the simple rule should be this: if collaboration is genuinely intended, it should be complete, and should allow collaborating European partners to influence not merely the development of star wars technology but also the use that is made if it. And otherwise, if collaboration is offered on terms that are less than full, it should be politely declined on the grounds that it cannot fail to be comprising. That is one version, but that there are others which are less polite is what some governments will be saying in Bonn. □

AIDS progress

Synthetic vaccine only a distant prospect

Atlanta, Georgia

THE virus responsible for acquired immune deficiency syndrome (AIDS) in humans may have entered the population recently by transmission from monkeys, according to a study of African green monkeys by Max Essex of Harvard University. Essex has found that apparently healthy monkeys show strong antibody recognition of STLV-III, a virus associated with immune deficiency disease in rhesus macaques which has many similarities with human AIDS virus. A simian origin for the virus would be consistent with new data suggesting that AIDS may have been present in humans of central Africa since before 1973.

Essex reported his findings here at the first international conference on AIDS, attended by two thousand researchers from 26 countries. The meeting was sponsored by the US Department of Health and Human Services and the World Health Organization, among others. The availability of two different primate models of AIDS, one with disease and one without, should be invaluable for development of an AIDS vaccine: comparisons between viruses might indicate those conserved regions that might be most usefully imitated in a synthetic vaccine produced by genetic engineering, so avoiding the problems caused by antigenic variability.

A vaccine that would prime the immune system to deal with the AIDS virus is one of the best hopes for dealing with the disease; it seems unlikely that a treatment will be found that will eradicate the infection once it becomes established in an individual, where DNA copies of the virus are incorporated into the host's own DNA. James Curran, head of the AIDS task force at the Center for Disease Control (CDC), said that if a vaccine became available it might be necessary to vaccinate all US citizens, starting first with those in high risk groups who at present show no evidence of infection. A vaccine is still several years away, however.

The latest data on the spread of AIDS in the United States are grim but unsurprising. By last week 9,608 cases had been diagnosed of which 4,712 have been fatal. Estimates of the number of US citizens who have already been infected with the AIDS virus, as indicated by the presence of serum antibodies, range from 0.5 to 1.0 million; more than 10 per cent of these are likely to develop the disease over the next five years, even if all further transmission of the virus stops immediately. The doubling period for the number of reported cases in the United States has increased slightly, but is still around 8–9 months. Principal conclusions from the conference have been reported to the US Department of Health and Human Services, and a separate report is being prepared for the World Health Organization.

New estimates have been made of the latency period between infection and the onset of disease symptoms, and it seems that the mean latency may be five years or even longer; this suggests that many people in high risk groups may already have been infected but are clinically unaware of the fact.

Between 70 and 90 per cent of haemophiliacs who receive regular infusions of factor VIII concentrate have detectable serum antibodies to the AIDS virus, and some data suggest that more than five per cent of these are likely to develop AIDS, with a further number developing "lesser AIDS" — AIDS-like symptoms that nevertheless fall outside CDC's definition of AIDS (Elaine Eyster, Pennsylvania State University School of Medicine). Estimates of the proportion of people with antibodies who will develop the disease are likely to go on increasing. Among one group of New York promiscuous homosexuals, the proportion of those with antibody that have developed AIDS is as high as 20 per cent (J Goedert, National Cancer Institute); it is not yet clear whether this high figure is due to some complicating lifestyle factor or whether it is because the virus has been present for longer in that group.

Very preliminary data from wives of haemophiliacs suggest that the AIDS virus is transmitted heterosexually by repeated contacts from male to female (J. Goedert, National Cancer Institute). A claim for female to male heterosexual transmission from prostitutes was also made (R. Redfield, Walter Reed Army Medical Center), though these data are also equivocal.

Some modestly encouraging results for treatment of AIDS with anti-viral drugs were reported, although none amounted to a major breakthrough. It is now apparent that neurological symptoms such as dementia are present in high proportion of AIDS patients, often associated with encephalitis.

US Secretary of Health and Human Services Margaret Heckler told the conference that AIDS is the chief health priority of the administration, but her speech, which studiously avoided mentioning the principal risk group, male homosexuals, did nothing to soothe the anger of homosexual activists and AIDS patients attending the conference.

Robert Gallo of the National Institutes of Health, who first isolated the virus known as HTLV-III, made a plea for less petty nationalism in the competition between research groups in different countries. Gallo's plea was not immediately effective: the AIDS virus continues to be referred to by at least three different names depending on the affiliation of the speaker, despite agreement that the virus isolated by different groups is basically the same, and despite an informal agreement to call the virus HTLV-III/LAV (or LAV/HTLV-III) in recognition of the work of the French group under Luc Montagnier at Institut Pasteur in Paris. The name issue is to be tackled later this year by the International Committee on Virus Nomenclature. Gallo also took many by surprise by calling for an end to the cult of the instant media authority.

All the blood banks in the United States are now screening donated blood for AIDS virus using enzyme-linked immunosorbent assay test kits manufactured by commercial companies, but implementing the tests has not been without its problems. According to Gerald Sandler of the American Red Cross, an anomalously high proportion of samples tested with the Abbott Laboratories' kit produced evidence of AIDS antibody on first testing in some areas of the country, although the proportion of repeatedly reactive samples was close to the expected 0.2 per cent. Samples that produce positive results on two subsequent tests are confirmed by Western blotting, but it is apparently impractical to carry out Western blots on all samples that produce a positive result just once.

It is also becoming clear that after infection by the AIDS virus, several weeks or months may pass before antibodies reach easily detected levels; any test that looks just for antibodies can therefore be at best an imperfect protection for blood banks. In Britain, initial investigations of the Abbott test have produced disappointing results, and it seems most likely that Britain will develop its own test for blood banks rather than importing US products; price is also a factor.

There is still no agreement on whether US donors who show clear presence of antibody that is confirmed by Western blot should be told of the results of the test. The American Red Cross says it is still reviewing options, and organizations in different states are following different paths. California has adopted a moratorium on conveying test results to donors until alternative testing centres are established where people can be tested for antibody without giving blood.

The fear is that as long as testing is available only at blood collection centres, people in high risk groups will be attracted to offer their blood in order be tested: questionnaires indicate that most people in high risk groups do want to knows whether they have circulating antibody, despite the uncertain prognosis. Some federal funds are likely to be made available to establish several hundred alternative test centres, though the amount is still being argued.

Tim Beardsley

Star wars

Israel attracted by spin-off

Rehovot

ISRAEL is almost certain to accept the invitation it has received from the United States to participate in the Strategic Defense Initiative ("star wars") research programme.

If the government does so, it will enjoy the support of most of the scientific community, which hopes that the project will provide research funds that are no longer available from other sources and also create positions for young scientists who might otherwise leave the country.

Professor Josef Singer, president of the Haifa Technion and an aeronautical engineer who has himself done defence research, supports Israeli participation but warns his colleagues that they should not expect the United States "to spend billions of dollars and to engage thousands of scientists and engineers".

Singer points out that the US armed forces have already spent millions of dollars over the past 30 years in support of research projects at institutes in Israel. "And", he says, "while Israel stands to gain more in resources and know-how, it also has a contribution to make. Otherwise, it would not have been invited to participate." He expects there to be benefits for Israel in the high-technology industries. And off the record, other Israeli scientists say that star wars research, although primarily aimed at finding a way to bring down Soviet ballistic missiles, may help to improve Israel's military potential closer to Earth. Specifically, they speculate about the development of an innovative deterrent that might compensate for the fact that shortage of money and manpower make it difficult for Israel to match the military build-up of its Arab adversaries.

A dissenting opinion comes from Professor Giora Shaviv, an expert on space research at the Haifa Technion, who dismisses the invitation to Israel as a propaganda move and says that Israeli researchers "have no real role to play" in the star wars scheme. A small number of politicians and academics have raised the subject of the moral and political implications of Israel's participation in the US programme.

Meir Stiglitz, who teaches a course on nuclear weapons at the Hebrew University's International Relations Department, claims that Israeli leaders are so blinded by the immediate benefits of cooperation with the United States in this sphere that they overlook the potential long-range dangers. As he sees it, these include the possibility that Israel may become a "desirable and legitimate" target for Soviet nuclear missiles, and might be attacked in order "to send a message" to the United States. **Nechemia Meyers**

Star wars

France rallies European forces

THE French government is determined to shake Europe awake, and to encourage all the nations of Europe to take a great leap forward in science and technology, by creating a new European research organization with considerable powers and funds, dubbed "Eureka". Or it may be that the French government is scared stiff of participation in the US Strategic Defense Initiative (SDI, or star wars) because it feels this might suck France dry of some of its best weapons technolgies, and wishes to form a common European industrial and research front. Eureka would be that front.

Which is the truer picture of French intentions? Probably both are true, which has made it a little difficult for Western European foreign ministers to respond to an urgent letter, proposing the flexible and rapid creation of Eureka, addressed to them last week by their French opposite number, M. Roland Dumas.

In the letter, Dumas says that Eureka (which in a rare French linguistic concession he gives the English name "European Research Coordination Agency") would set up major programmes half-funded by Eureka and half by industry in optronics, new materials, high-powered lasers, artificial intelligence, high-speed and ultra-miniaturized microelectronics and space. Any European state should be able to join in any programme, Dumas suggests, leading to a "variable geometry" Europe for research. But what research? Dumas' shopping list is remarkably similar to one that might be drawn up by SDI, rather than the new technologies in general (where is biotechnology, for example?), but the letter does not mention SDI by name.

Nevertheless, French interest in creating what the Prime Minister, M. Laurent Fabius, calls "a European area for research" is well known, and long pre-dates interest in SDI. France has doubled its government civil research spending since President Mitterrand came to power in 1981, but now realizes that France cannot go it alone against the United States and Japan. Thus Pierre Papon, director-general of the principal French research council, the Centre National de la Recherche Scientifique (CNRS), which has 10,000 researchers, made a detailed tour of Europe last year seeking bilateral research agreements, and the present research minister, ex-president of the French space agency the Centre National d'Etudes Spatiales (CNES), has also shown himself a dedicated European.

But these individuals and others in the French science political scene may be getting impatient at the pace of European integration in science and technology. ESPRIT, the European programme of research in information technology, flagship of the European Economic Community (EEC) programme in integrated "pre-competitive" research, has been extremely slow to spend real cash, and for the moment can claim only that it has brought a few European companies to the same table. The only really working institutions of any size seem to be the European Organization for Nuclear Research, CERN, which has only a moderate economic impact, the European Space Agency (ESA) of which Curien has been chairman, JET (the European fusion experiment, with impact only far in the future) and the European Airbus.

France wants much more, and earlier this month Jacques Delors, the new French president of the European Commission (the EEC bureaucracy), tried to provide it. In a written presentation, he asked European ministers at a summit meeting to double EEC research spending from its present three per cent of the Brussels budget to six per cent, and he linked the increase with the need for Europe to respond to SDI. Ministers — who at present in the 10-nation EEC must agree any proposal unanimously — threw out both ideas. Many states were, it seems, prepared to increase the research budget, but by nothing like the factor of two, and the SDI link was described by some participants as "half-baked", rushed and ill-prepared. There seems to have been no collaboration between Delors and the French government over the issue, and it may be that Delors had had wind of the Eureka proposal and wished to put up an EEC stake, fearing the likely weakening effect of Eureka on Commission power in research.

Nevertheless, Delors was unsuccessful, and now Dumas has proposed Eureka, determined, it seems, to see something happen for European research. But the announcement of his initiative last week in Paris was not made through the usual channel, the Wednesday council of ministers meeting, but direct from the ministry of foreign affairs itself. Staff of CNRS "read about Eureka in the press". There seems to have been "a little problem of communication" between the foreign ministry and the research council, though Pierre Papon "has since become involved in discussions".

Moreover, the links with defence have clearly become stronger, as Dumas' letter to foreign ministers was delivered a few days in advance of this week's meeting of the Western European Union, a recently-revived forum in which seven of the major European states discuss defence matters. The letter is intended for discussion "on the margins" of the meeting. Dumas will no doubt be expressing concern about French industry, in which spending on armaments has become the principal component. Last year, for the first time, France exported more arms to the United States than it imported from that country. And

recently a number of French companies including Thomson, the nationalized electronics company, have gone well into the black through trade in armaments, much of it to the Middle East. Often this trade relies on high technology weapons, such as Exocet, the missile which was so dangerous to British ships in the Falklands conflict. France does not seek a one-way drain of expertise or experts to the United States through premature involvement in the star wars programme.

Thus Dumas is concerned by the position of Britain, where foreign minister Sir Geoffrey Howe has criticized SDI so strongly (on strategic grounds) that France fears Britain may not participate at all. Dumas is slightly happier with West Germany, with which France has increasingly been developing a special relationship in Europe. There, Chancellor Kohl has expressed full support for Eureka but has gone further than the French by agreeing separately with the United States to take part in the SDI research programme. Kohl will send a team of negotiators to Washington to discuss the matter, he has told the Bundestag.

Some united participation in SDI is what France wishes to see. And it is even preparing its public SDI is "star peace", not "star wars", defence minister Charles Hernu claimed on French radio last week. "And France must have its place in this star peace", he proclaimed.

Robert Walgate

Star wars

US pressure on Japan

Tokyo

THE US Strategic Defense Initiative ("star wars") seems to have found a fan in Japan's Prime Minister Yasuhiro Nakasone, who has been extolling its virtues in recent Diet speeches after his meeting with President Ronald Reagan in Los Angeles earlier this year. Pressure for Japan to make its support official is being kept up by a series of visits from US experts. The real issue, however, is how far Japan is prepared to move from its pacifist constitution, which prevents participation in collective military efforts.

At the end of last month, US Defense Secretary Caspar Weinberger formally asked Japan as well as other US allies to inform the United States within 60 days whether it is willing to participate in the US research programme for the Strategic Defense Initiative (SDI). Weinberger's request was immediately followed up by a series of visits to Japan by US military experts and a delegation from the North Atlantic Treaty Organization (NATO) to "explain" SDI and to look for areas of cooperation.

Although the 60-day deadline has subsequently been dropped by the United States as a bureaucratic "error", further teams of US experts are expected in Japan, starting this week.

Japan's space technology is of course still relatively backward and largely developed under US licence. But where Japan has something to offer is in its electronic communications technology, particularly in the use of extremely-high-frequency microwaves to transmit huge quantities of data from satellites to the ground.

Japan's defence agency has, in fact, already completed a study of SDI aimed at assessing the efficiency of both chemical and nuclear-pumped X-ray lasers. Although the study seems to say only that enormous improvements in aiming accuracy would be required to shoot down intercontinental ballistic missiles, that it was carried out at all is an indication of the extent of Japanese interest.

In theory, Japan should have no military forces at all; Article 9 of the constitution (forced on Japan by the United States at the end of the Second World War) says that "land, sea and air forces, as well as other war potential, will never be maintained". In fact considerable "self defence" forces have been in place for years. But until very recently the Japanese government has regarded supplying military technology to foreign countries as going much too far. In 1983, however, considerable pressure by the United States forced Japan to accept a bilateral agreement allowing the transfer of military technology to the United States — again, US interest lay largely in the communication field.

Star wars would be another step up in Japan's military involvement with the United States, but would be seen as a step onto a slippery slope by opposition socialist parties. Government representatives have already had to resort to semantic juggling to make a possible involvement respectable. Military technology transfer is as yet possible only to the United States, but SDI would probably involve other countries. The Foreign Ministry's Treaties Bureau chief Hisashi Owada sidestepped this issue by saying Japan can help the United States with the research, "even if NATO countries take part in it, so long as military technology supplied to the United States remains there".

If SDI research does go ahead, it will bring closer the day when Japan's giant corporations can go into the military export business. But Nakasone may find there is more opposition to the plan from the general public than he has bargained for. Like other world leaders, he has found it easier to agree with Reagan when having a cosy face-to-face chat than to sell Reagan's ideas back home. Nakasone is scheduled to have his next get-together with Reagan on 2 May, just before the Bonn summit, and star wars is high on the agenda.

David Swinbanks

Spain

Law of Science brings change

Barcelona

SPANISH research institutions are likely in the next few months to face a similar upheaval to that experienced in the universities as a result of the Law for University Reform (see *Nature* **312**, 8; 1984).

The Spanish government has now approved the Law of Science (Ley de Fomento y Coordinacion General de la Investigacion Cientifica). The bill has been sent to parliament, where the Socialist Party has an absolute majority in both houses, and a vote is expected before the end of the year. Government approval of this law, after months of discussion between different ministries, may represent an important step in the reform of the organization of research in Spain.

The Law of Science aims to establish mechanisms to define research priorities, to programme the resources for research and to coordinate the work of those in universities and research institutes. The bill also aims to redefine the role of the main public bodies concerned with research, which until now have often had overlapping functions because of the accumulation of 50 years of contradictory legislation.

Under the new law, the National Plans for Research will define research priorities and determine how public funds should be distributed. The draft of a National Plan for Research and Development for the next five years has also been presented as an annex to the law. Subjects to which it would give priority include microelectronics, biotechnology, transportation technology and high-energy physics. Fellowships tenable both in Spain and abroad are intended to double the number of researchers in industry and public organizations within five years.

The Law of Science also proposes a new structure for the main public research bodies, including CSIC (Consejo Superior de Investigaciones Cientificas) and JEN (Junta de Energia Nuclear), to be called the Centre for Energy, Environment and Technological Research. They will be organized so as to promote the mobility of staff between different institutions.

The need for a change in the regulation of Spanish research has been generally acknowledged. Officials in the Ministry of Education and Science believe the new law may help to break down the barriers between people doing the same work in different departments, to promote interaction between public and private research and to continue the trend towards better scientific control and less bureaucracy in the administration of public resources. If these reforms are coupled with increased public and private spending on research, the law will enhance Spain's contribution to science and technology.

Pedro Puigdomenech

Cancer research

France moves ahead

FRANCE is moving ahead of Britain in cancer science, according to a literature review* carried out by Jean-Claude Salomon and a colleague at the Institut de Recherche Scientifique sur le Cancer at Villejuif near Paris.

Salomon based his studies on the 300 or so key papers selected annually for summary in the *Year Book of Cancer*, a regular series of reviews of the world cancer literature produced for the education of US doctors. The yearbooks, edited at the M.D. Anderson Hospital and Tumor Institute in Houston, Texas. "satisfy certain criteria of rigour and objectivity", says Salomon, "though they may perhaps favour American authors". The editorial board of 155 discipline specialists contains representatives from 14 countries, but most members of the board are American. Each year, this board selects the most significant papers from among around 50,000 published each year in 1,200 major journals for summary in the yearbook.

Using this source as a measure of international productivity of cancer science (including clinical papers), Salomon discovered that from 1965 to 1982, the proportion of papers summarized in the yearbooks that were British had declined from 8.3 per cent (the then highest figure outside the United States) to 4.2 per cent, whereas French articles had climbed from 2.3 per cent to 4.6 per cent, putting France in the role of leading nation for cancer research outside the United States.

The founder-editor of the yearbooks (and founder of the M.D. Anderson Tumor Institute), Dr Randolph Lee Clark, believes these figures may well represent real trends. While France had seen fit to invest in 25 "cancer centres" during the period reviewed, the British scene had been almost stagnant, he observed.

Salomon's figures also show a poor result by West Germany and by Israel, each with under one per cent representation in the yearbooks.

In France itself, the clearest signal from Salomon's review is that there has been a strong shift in cancer science from Paris to the regions. Between 1965 and 1976, three-quarters of French articles summarized in the yearbooks came from Parisian institutes. But from 1978 to 1982, Paris could claim little more than half, a result with statistical significance at the one-in-a-thousand level, according to Salomon. In particular, the renowned complex of institutes at Villejuif, including Solomon's own workplace, which had more than 40 per cent of the articles in the first period, had only 12 per cent in the second. This may be a measure of the effectiveness of a big French effort at decentralization 12-15 years ago.

Could there be bias in the selections? Dr Clark claims not, and that he tries to ensure that "there are no national barriers "in the coverage of the yearbook. The sole British representative on the editorial board, Dr Neils Atkin, cytogeneticist at Mount Vernon Hospital, Middlesex, described Salomon's observations as "rather sad" for Britain and added that "I can't think of an explanation that would give us any relief". The editors try to be objective, he said. **Robert Walgate**

* Salomon, J-C & Blanchin, P *Bull Cancer (Paris)* 71, 382-387 (1984)

Heavy ions

Darmstadt to leapfrog Berkeley

Hannover

THE heavy-ion laboratory at Darmstadt, south of Frankfurt, last week received its reward for its discovery of element 109 three years ago — DM275 million (£65 million) with which to build a bigger and better ion accelerator.

The West German federal minister for research and technology, Dr Heinz Riesenhuber, announced on Thursday that the laboratory would be allowed to go ahead with a plan it had nurtured for at least the past four years. The scheme is to feed ions from the existing linear accelerator UNILAC, at an energy of up to 20 MeV per nucleon, into a synchrotron designed to yield energies 100 times as great. There is also to be a storage ring equipped to bunch and to homogenize the velocity of heavy ions from the synchrotron.

Those working at the laboratory are ecstatic about the prospect. While the maximum energy from the new machine is not very different from that of the similarly constructed BEVALAC at Berkeley in the Unites States, the intensity at Darmstadt will be greater by a factor of 1,000.

The search for other exotic nuclei continues at Darmstadt, even though people talk less confidently now than a few years ago of a possible "island of stability" in the region of atomic number 116 where heavy nuclei would be relatively stable. Indeed, the existing linear accelerator has just finished a two-week run looking for signs of element 110 in the collision of nickel and lead ions. But people now insist that the intended machine will be justified even if no further heavy elements are found.

One field of interest is the beta-decay of heavy nuclei stripped entirely of their orbital electrons. Collisions at 2,000 MeV per nucleon may also simulate the condition of nuclear matter, as in neutron stars, while there is virtually the whole of the atomic structure of heavy ions with many charges to be explored. On the technical side, there are interests in the use of heavy-ion beams for pumping X-ray lasers, and for investigating the properties of dense cold plasmas (which may be crystalline).

The plan is that the extension of the Darmstadt laboratory should be completed in the next five years. The laboratory hopes to increase its staff (now 450 with 50 supernumaries), although not by the whole of the 70 positions asked for. Of the extra funds now made available, 90 per cent will come directly from the Federal Ministry of Research and Technology, while the remainder will be divided (in the ratio 9:1) between the federal government and the *Land* of Hesse. The laboratory's budget is now DM 80 million a year.

John Maddox

Director for Newport News facility

Washington

HERMAN Grunder of Lawrence Berkeley Laboratory in California has agreed to assume on 1 May the directorship of the Continuous Electron Beam Accelerator Facility, the machine that may be used to study "bags of quarks" and that is to be built in Newport News, Virginia, by a consortium of south-eastern universities led by the University of Virginia.

Grunder's decision to accept the position is seen as a major step toward legitimizing the project, which has been plagued with political and technical problems from its start two years ago.

First there was a major political battle with Argonne National Laboratory over who should build it. Although the Department of Energy Nuclear Science Advisory Committee (NSAC), traditionally the last word of the physics community, endorsed the Virginia group's proposal, Argonne broke protocol and appealed directly to the Secretary of Energy, enlisting the aid of the Illinois congressional delegation. In the process, enough questions were raised about the technical expertise of the Virginia group, the technical feasibility of its design, and the accuracy of its cost estimates (which have already grown from the $100 million figure presented to NSAC to more than $200 million in congressional testimony last year) that a sceptical Congress insisted on a go-slow policy. Construction funds requested in the very first year have yet to be approved. The resulting lack of confidence in the physics community that the project would ever in fact begin made recruitment of a director difficult.

Meanwhile, though, the accelerator received a much-needed boost when NSAC, asked by Congress to re-evaluate the project, gave it a firm reendorsement.

Stephen Budiansky

Bhopal aftermath

Legal complications mount

Washington

THE lawsuit filed in New York two weeks ago by the government of India on behalf of victims of last December's disaster at the Bhopal Union Carbide plant has placed on the table a complex series of legal issues that will have to be resolved before even an out-of-court settlement can be reached.

Two of the thorniest questions are whether the US district court will allow the case to proceed in the United States and, if so, whether Union Carbide will be able to countersue the Indian government. To be resolved, too, is the validity of India's claim to be the exclusive representative of the victims and the status of nearly 1,000 lawsuits filed by victims in Indian courts.

In preliminary hearings last week, Judge John Keenan refrained from showing his hand on any of these issues. But, invoking the broad discretionary powers accorded federal judges in the United States, he urged Union Carbide to make an immediate contribution of $5-10 million for emergency relief; Union Carbide the next day agreed to turn over $5 million. Both Keenan and the company made clear that this was not an admission of liability (or even of the US court's jurisdiction).

From the start, the Indian government has said it intended to sue Union Carbide in the United States. Although any compensation awarded by US courts for lost wages, for example, would be based on Indian standards, US courts are much more generous in their compensation for less tangible losses, such as pain and suffering, than are their Indian counterparts.

Awards in US courts are set by juries, usually more sympathetic to victims; Indian cases are heard by judges only. And Indian judges have been very reluctant to award punitive damages, except in cases of clear deliberate wrongs. US courts generally allow punitive damages against defendants who have shown reckless disregard for safety, even when not deliberate.

If Keenan agrees to hear the case, the pressure on Union Carbide to settle out of court will increase measurably. The company has already made one offer of settlement, unofficially put at $200 million, an amount reportedly equal to its insurance coverage. The Indian government has rejected that offer.

If the case is tried in the United States, the company would be facing the real possibility of a judgement of perhaps $1,000 million, approximately one-tenth Union Carbide's entire net worth. The pressures on the company for a settlement are already considerable in any case.

But meanwhile Union Carbide is applying some legal pressures of its own to urge concessions on the other side. The compay's lawyer has made it clear that he will argue that the case should be heard in India as the more appropriate forum. He has also suggested he may move to have the US law firm retained by the Indian government disqualified because it once represented Union Carbide, though on an unrelated matter. And there is also the threat of a countersuit against the Indian government. Normally, governments enjoy "sovereign immunity", and cannot be sued for their actions. But under US law, a foreign country that brings suit in a US court cannot claim sovereign immunity in defence against counterclaims. Union Carbide would probably argue that by allowing squatters to settle near the Bhopal plant in defiance of municipal plans, the government shares responsibility for the injuries.

A wild card in any resolution of the claims is who has the right to represent the victims. The Indian government has enacted a law declaring the government the exclusive representative, while allowing victims to hire their own lawyers who could join the government's action. One faction of the US lawyers who signed up victims — a faction that has been hoping to organize the case into a class action — is challenging the Indian law in Indian courts. It is being joined there by Indian lawyers whose suits filed against Union Carbide in India have been frozen by the law.

Stephen Budiansky

Handicapped infants

Physicians win the day

Washington

THE furore that greeted the Reagan administration's regulations for the treatment of handicapped infants seems finally to have been quelled. Last week, the Department of Health and Human Services (HHS) issued new rules that for the most part defer to the physician's judgement in deciding how to treat such infants and that keep the federal government out of the enforcement business.

The new rules, the result of an arduously crafted congressional compromise last fall, require only that each state's child abuse agency should develop procedures for investigating and "responding" to reports of medical neglect of infants and that they have the legal authority to seek a court order to prevent "withholding of medically indicated treatment". The rules do not apply to cases in which the treating physician believes, in his "reasonable medical judgment", that:

- the infant is in an irreversible coma;
- treatment would merely prolong dying, fail to correct all of the infant's life-threatening conditions, or otherwise be futile; or
- treatment would be virtually futile and would itself under the circumstances be inhumane.

Earlier versions of the rules stirred great resentment among paediatricians and hospitals. Under the original rules, issued in the spring of 1983, hospitals were required to post prominent signs warning that "discriminatory failure to feed and care for handicapped infants in this facility is prohibited by federal law".

The rules also established a "Baby Doe hotline" for receiving reports of medical neglect; HHS set up an investigative team that would be sent out on short notice to examine these reports. The hotline received about 400 calls, most of them from cranks; in the four cases examined no violations were found. But critics complained that the parents of the infants and the hospitals had been put through a needless ordeal. The regulations had been in effect for only a month when they were thrown out by a federal judge acting on a lawsuit brought by the American Academy of Pediatrics and others.

The physicians' organizations also argued that the two well-publicized cases were carefully scrutinized by state authorities, and in both cases local courts refused to intervene. Almost all states include medical neglect in their definition of child abuse; courts have often intervened in cases where parents have refused to allow treatment for their children on religious grounds. The physicians' group thus argues that no federal involvement is necessary.

The new rules indeed go a long way to acknowledging that, and make clear that the federal government is not to establish standards prescribing appropriate treatments. In ordering HHS to issue these rules, Congress in fact seemed chiefly concerned with defusing the issue in such a way that the administration — and the right-to-life groups that the administration had been appealing to in issuing the original rules — could save face. Physicians' organizations remain concerned, however, that even the new rules will put improper pressure upon paediatricians always to choose heroic, aggressive treatment for fear of being accused of withholding treatment.

HHS says it received 116,000 letters commenting upon a draft version of the new rules, published in December. One major change that HHS made from the draft to last week's final version was to remove a set of definitions of terms from the body of the rules into a non-regulatory appendix. Physicians said the definitions of such terms as "life threatening condition" included specific examples and placed the federal government in the position of dictating specific procedures. The six principal Senate sponsors of the compromise bill urged the agency to consider these complaints seriously, noting that there was a danger of undoing the careful balancing that went into the bill.

Stephen Budiansky

US universities

All change at St Louis

St Louis, Missouri

THE Washington University, part of this city's pride (after the arch across the Mississippi and its baseball team, the Cardinals), is part way through an administrative revolution that keeps many of the faculty awake at nights. Two years ago, the trustees decreed that the School of Arts and Sciences should follow the professional schools into financial and administrative autonomy. One result could be that academic departments less able to fend for themselves financially — philosophy but even physics — might go out of business.

The School of Arts and Sciences has had autonomy thrust upon it. Two years ago, it was the only school of the university not paddling its own canoe. The argument for change starts from the observation that other schools that have been independent for some time (medicine conspicuously) have prospered, partly from the ease of making deals with outside backers and partly by commanding the loyalty of their alumni. But then, as the administrators are fond of saying, is this not what the Harvards of this world do?

Under the arrangement, called "being put on reserve" in reference to the share of the whole endowment that autonomy commands, a school is the fiefdom of its dean. Policy on admissions, curriculum and even salary levels is determined separately. Now, in the School of Arts and Sciences, tuition fees (in excess of $8,000 a year for undergraduates) go into a pool with other income, but the school pays a levy to support central activities.

Worries among the faculty take several forms. The philosophy and classics departments (large and small respectively) may not be financially viable, but can a university claim the name without offering courses in such fields? But even physicists fear that the engineering school, which now pays a fee to arts and sciences for service physics teaching, may decide to teach its own physics as an economy. (The chancellor, Dr John Danworth, says the service fee, fixed by the trustees, could not be changed without their consent.) Then there is the general worry that autonomy will turn all academics into fund-raisers, or that only successful fund-raiders will prosper.

The chancellor is anxious that autonomy should work, but is for the time being open-minded about the change. If it does not bring the benefits intended, the trustees would think again. And a successor might wish to see the omelette unscrambled. But in Danworth's view, only autonomy can make a faculty responsible for its academic future. He cites his own earlier success, as head of the medical division, in turning a bankrupt dental school into a successful enterprise with the help of a tapering subvention from the centre ($600,000 over three years) coupled with the threat that failure would spell closure.

In any case, Danworth says, it is no longer possible for the head of a private university, forever occupied with fund-raising and negotiations with government agencies, to be so involved with academic life as to provide single-handed academic leadership. This leads him, after careful thought, to an unyieldng position on the issue of the weaker departments; if the faculty of arts and sciences should think of closing one of them, the whole value of independence would be lost if he or the trustees should intervene to halt the closure. But what a chancellor can do is to fire a dean who has failed to carry the faculty with his decisions. Danworth is said to read faculty meeting minutes like a hawk.

Ultimately, the success of the experiment will hang on whether arts and science can raise the funds needed to stay alive, and on whether the faculty is sufficiently committed to the ideal of a liberal education for the well-to-do to indulge their less commercially viable colleagues.

That money is so dominating surprises nobody at St Louis, although most academics are more concerned with the competition for high-quality students, which they say is a prerequisite for commercial success. They point to rising scores in the national college boards examinations and a widening catchment area (with only 20 per cent of undergraduates from the locality). Physics has now followed engineering in offering a handful of undergraduate scholarships, saying that even students who compete unsuccessfully for them leave their applications in. The faculty also claims that other universities are prevented by the egalitarian climate from following this elitist course.

So far, the university has been lucky in its donors. The chemical company Monsanto, only one of the reasons why East St Louis (across the river in Illinois) is such a slum, made a 5-year grant of $27 million to the medical school two years ago, from which non-medical biologists also profit. McDonnell Douglas, the aircraft manufacturer, has been generous over several years, partly because of the interest of the late founder of the company (known generally as "Mr Mac") in space research (whence the flexibly endowed McDonnell Center for Space Research) and in parapsychology (engendering less pride). By contrast, General Dynamics, also based in St Louis and now in trouble for misdemeanours such as lodging a senior manager's dog in a commercial kennel at the Pentagon's expense, is almost conspicuous for the modesty of its support.

As a research university, Washington dates its rise to the post-war chancellorship of A.H. Compton, who arrived at St Louis in 1945 with a group from Los Alamos. More recently, the medical school has made the running.

For a big-city urban university, Washington is suprisingly well-provided. It is compact (mostly on one campus, with the medical school two miles downtown) and hemmed in by amenities such as Forest Park, the site of the world's fair in 1904 which persuaded the rest of the United States that St Louis is not some outlying suburb of Chicago (more than 300 miles away). The setting is also a physical constraint on growth, but nobody wants that any more. The snag is that even successful departments may be smaller than a critical intellectual mass which is why, whatever the consequences of autonomy for arts and sciences, a measure of concentration may be prudent.

John Maddox

European geoscience union thrives

THE European Union of Geosciences (EUG), at its third meeting in Strasbourg on 1-4 April, appears to have established itself. Since EUG's foundation five years ago under C.J. Allègre of the University of Paris, attendance at the biennial meetings has increased from 600 in 1981 to more than 1,500 this year. The annual spring and fall meetings of the American Geophysical Union attract between 2,000 and 2,500 participants.

The need for such a forum in Europe was one of the stimuli for the creation of EUG, says the new president, Professor E.R.Oxburgh (University of Cambridge). There is a particular need, he says, for European earth science research students to meet and to present their work to more established people. To this end, costs are kept as low as possible. This year, more than 200 travel grants, valued at £10,000, were awarded for the meeting. In his presidential address, H.Wänke (Max Planck Institute, Mainz) said that he knew of no comparable organization that spends so much of its income in this way.

EUG works on the principle that membership is strictly individual. Other than a part-time secretary provided by the European Science Foundation, the union has no permanent administration. Allègre says that EUG is essentially anti-bureaucratic.

Meetings are organized in turn by members from one country. The 1985 meeting was arranged primarily by the University of Cambridge, and the next(in 1987) will be organized by Eidgenossische Technische Hochschule, Zurich. After that, Italy will probably take over. All the meetings will take place in Strasbourg. Although some officers feel that this rotation of responsibility is a strength, others fear that growth may make a more permanent administration inevitable.

Peter Gambles

Hannover trade fair

Something for everybody

Hannover

THE world's largest and glossiest trade fair has become a supreme test of endurance. While the organizers boast of having crammed 60,000 cars into the car parks, yawning salesmen excuse themselves by saying they have just spent three hours in traffic.

Inevitably, innovation tends in these circumstances to be deeply buried in the products that people want to sell, preferably on the spot. But, after a week's spring sunshine, solar cells made of amorphous silicon abound, making enough electricity to circulate water in a toy swimming-pool or to light mock traffic lights. People like Interatom, a subsidiary of the nuclear energy constructor Kraft-Werke Union, say the opportunities for special uses are now rapidly increasing, the uneconomic character of solar cells notwithstanding.

Hannover's research ghetto, a building with the subtlety and floor-area of an aircraft hangar, is lavishly sprinkled with displays by West German universities of all kinds (of which there are three), most of them seeking industrial backing for research begun with public funds in academic laboratories. The phenomenon is partly instinctive and partly federal and local (*Land*) government policy. Industrial exploitation has become the watchword. (Some say it always has been.)

Academic stallholders hope to benefit in several ways. Professor Bert Kuppers from the Fachhochschule at Aachen (a technical college not to be confused with the university at Aachen, called the Technische Hochschule) has a model of a generator capable of taking the variable-speed output from a windmill and producing constant-voltage 50 Hz current; if he is able to find a backer before the week is out, he will be in business.

Others look for industrial support to help win government grants. Professor Gerhard Muller from the Gesamthochschule at Essen, for example has developed a technique for coating objects made of materials such as plastic with metals of almost any kind. The principle is to use materials evaporated from an anode both as the working gas for a discharge and the coating substance. The development has cost the university less than DM 50,000 so far, but if the fair can recruit enough industrial testimonials, the chance of winning a big government grant will be bright.

On the biological front, pollution control and tree death are the prominent issues. The University of Oldenburgh has constructed a prototype of a self-contained system for processing sludge from sewage works which yields an oil-substitute rich in aromatic hydrocarbons and runs on energy by burning waste gases, so that the return of waste to the environment is kept to a minimum. (The one per cent of residue contains tightly-bound metals, which may yet be reclaimed.) This is a project supported so far by the government which now needs industrial support.

The Karlsruhe nuclear research centre is on a similar tack with a pilot plant for burning rubbish in a self-contained environment. Heat makes electricity, and waste acid gases in solution can be used in reclaiming heavy metals from the solid waste.

Tree death is West Germany's anxiety just now, with the lumber industry an important part of the agricultural economy. The University of Freiburg and the Karlsruhe centre (the Kernforschungszentrum) have both developed techniques for identifying damaged trees from infrared photographs, and the use of these methods has already helped to suggest the sources of damaging pollution on the Western edge of the Black Forest. And people are growing trees in controlled test chambers, as the Gesellschaft fur Strahlen und Umweltforschung in Munich is eager to proclaim, so that the physiological and biochemical effects of the various pollutants can be investigated individually.

This is the plentiful small change of the research on display, which is most of all impressive for the diversity and ingenuity of the academics. At the other end of the scale, the major research establishments under the umbrella of the Arbeitgemeinschaft der Grossforschungseinrichtungen (big science spelled out) are also eager that passers-by shall recognize the useful things that they can do.

Radio-frequency resonators built as means of feeding power into particle accelerators (as at DESY, the Hamburg accelerator laboratory) are offered as being capable of other jobs. The Darmstadt laboratory (which accelerates heavy ions) also offers its beams as a way of punching micro-holes in pieces of material, if a little unconvincingly.

This group of a dozen national laboratories is exceedingly diverse and a little uncertain about its future. In the last year of the five-year government demand that spending should be reduced by 1.5 per cent a year, the organization has been cheered by the federal government's decision (see page 662) that Darmstadt should expand. But there is now some dismay that the umbrella organization should have been told, a few weeks ago, that it will have to make reductions of spending by differentiating among the twelve establishments, which range from the cancer research centre at Heidelberg to the national aerospace organization which is scattered over West Germany. People fear this new policy as a recipe for internecine strife in what was previously a self-contented organization.

The federal minister for research and technlogy, Dr Heinz Riesenhuber, gave away nothing on the degree of economy expected when he popped in by helicopter last week. (Helicopters land in the car park, whence ministers are ferried between cars full of machine-gun-toting police.) The minister shook hands or patted heads as appropriate, and held a public long-distance conversation of conspicuous banality with the West German research station in the Antarctic before being whisked away.

The Antarctic programme is nevertheless big — the annual budget runs at DM 65 million a year. One of its more intriguing sets of observations is that of the tides on the ice shelf at the entrance to the Weddell Sea, from which it is hoped eventually to disentangle simple ocean tides and those affecting the floating ice.

Industrial research is prominent, although electronics research is curiously inconspicuous. (Nobody has a stand devoted to the making of silicon chips.) The West German love affair with the motor car is illustrated by the large crowds around the Mercedes-Benz and Volkswagen stands. Preussac, the minerals extraction company, has a vivid exhibition of ores dragged up from the sea-bed, but is not especially hopeful that it will make money from its interest.

For the rest, the fair's technology is a curious contrast between the machines with moving parts and those which seem to have none. (The halls housing the mechanical machines are, by comparison, as quiet as cathedrals.) This, no doubt, is one reason why the organizers think they can safely split the fair next year. Most machinery-makers show something very old — Thyssen, for example, has a coal-burning locomotive outside its stand.

The computer people, by contrast, are all bustle. Apple, the US computer maker, has the best crowd-puller — it has simply provided 60 machines with which people can play if they fight their way to the head of the queue. Toshiba's illusionist is also a draw, but is there merely to suggest that his sponsors' photocopiers are magical. Given the time and energy, a person could decide which system will best meet his needs, although the computer-watchers are chiefly interested in computer-aided design and the like, which goes to show that this fair has become an essential part of West Germany's system of technical education.

Not everything is on this high plane. The lighting-hangar does give some manufacturers of specialized lighting systems a chance to show off, but it is mostly a display of lampshades of the kind that one might find (but would prefer not to find) in one's living-room. And an Australian company is selling (like "hot cakes") audio tapes of the sound of the ocean waves in which, it is said, there are hidden messages that will subliminally allow one to become better-adjusted in one of a dozen specific ways. How to live with traffic jams might be a good title for next year. □

Embryo research (contd)

SIR — The status of the early human embryo has been background music for much dancing on the head of a pin. The latest example is provided by C.B. Goodhart (*Nature* 14 March, p.126). He finds justification for "use of IVF embryos for experimental work expected to result in death" in the following argument. The fertilized egg *ex utero* is like an unfertilized egg in that voth require external action to realize their potential to become an adult, the fertilized egg requiring activation by a sperm (or a substitute). But, the argument continues, fertilization does not really change "right to life" status because that right relates to being human and alive — which is characteristic of both gametes and zygotes. *Ergo*, since zygotes and early embryos *in vitro* will, like unfertilized eggs *in vitro*, die without external help, both are ethically available for experimentation unto death.

After vertigo subsides, one asks why the discovery does not apply to zygotes or early embryos in normal reproduction. Easy — "the embryo *in utero* has of itself (*sic*) the full potential to complete its development without any further intervention from outside". Possible parallel — the bank robber, once inside the bank and having cowed the cashier, has earned his ill-gotten gain because he can (barring police interference) carry it outside without further external aid.

But now, in truth, the embryos (blastocysts) *in utero* and *ex utero* have exactly the same potential, as demonstrated by normal development of *ex utero* blastocysts when transferred to a physiologically prepared uterus. Moreover, the state of both is very different from that of an unfertilized egg by virtue of the *genetic* individuality conferred by gamete fusion.

If there are good reasons why IVF embryos should be available for clinical investigation (and I believe there are), the justification is better provided through contemporary ethics and science, rather than from a return to scholasticism.

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SIR — C.B. Goodhart's argument (*Nature* 14 March, p. 126) that embryos fertilized *ex utero* lack independent viability and therefore are not potential human beings like their brothers and sisters in the womb after natural conception, is at first glance attractive, in that it might allow one for instance to be anti-abortion yet pro-experimentation on very early embryos; but it involves the debatable assumption that what comes naturally is outside our control in the sense that we can interfere with it only negatively, whereas for an embryo fertilized *in vitro*, positive intervention is required for its survival. Is this a legally or morally valid distinction, especially for physicians and surgeons dedicated to interference with nature and rightly regarded as culpable if they do not do so when asked in the supposed interests of a patient, and might not feminists enquire whether their sex must regard themselves as the passive and vegetative receptacles of an embryo implanted in their womb by any male (or even by the Holy Ghost) who succeeds in depositing in them the necessary seed? Surely it is time we left the suspect word "natural" to advertisers of semisynthetic foodstuffs and took it upon ourselves to live up to the very worrying responsibility that we have for ordering our own destinies; or do we no longer assume that what man does is no longer part of nature since he is no longer mainly concerned with adaptation to his environment, but can adapt it to himself?

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Value-laden science

SIR — M. Hammerton's letter (*Nature* 31 January, p.343) demonstrates unwittingly that it is more difficult to identify value-free science than the unwary might realize.

The statement which Hammerton believes to be value free, "The lethal dose of cyanide is x gm/kilo body weight", contains implicit value judgements in the definition of "lethal dose" and how this relates to the real world, for example a coroner's inquiry. Is the lethal dose for a particular human individual determined better on the basis of extrapolation of LD_{50}s from animal experiments, or from a smaller sample of mortality data collected from other humans? Furthermore, independent of the definition of "lethal dose", if the forensic evidence indicates that the victim has a body burden of $0.2x$ gm/kilo, was cyanide the cause of death? Whatever the body burden of cyanide, what other factors ignored by the forensic evidence (such as the psychological state of the victim) could be relevant to the death? To answer such questions, value judgements are unavoidable.

Ironically, the statement which Hammerton believes is value-laden, "I am going to poison my spouse", can be interpreted as a statement of pure intention or as a prediction of one's own future behaviour. With these interpretations, although the statement is unpleasant, it contains no ethical, social, political or other evaluative judgements, and so can be considered to be value free. To make it clearly value-laden, one could re-interpret it as "Rather than (say) shoot my spouse, I shall use poison", or one could modify it to "It is good/socially desirable/profitable to poison my spouse".

Although the matter is controversial amongst philosophers of science, people might wish to hold a "pure" mathematical statement, such as $2+2=4$, is value free in its content. However, even then the context could in some cases make it value laden: for instance, if it represented a reductionist approach to a scientific problem which some researchers believe can only be treated adequately by a holistic approach.

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Sizewell inquiry

SIR — Your leading article (28 February, p.723) on the prospects for UK energy policy following the ending of the Sizewell inquiry and the coal industry strike raises a number of important questions. Unfortunately, it would demand too much space adequately to respond to all of them. I wish therefore simply to point out one factual error rather than to take issue on matters of interpretation.

You state that part of the explanation for the extreme length of the Sizewell inquiry (340 sitting days taking just over 2 years) was the inspector's decision at the outset to allow documentary evidence to be accepted only if it was read aloud, but this is not true. The inspector did require the proponent party, that is, Central Electricity Generating Board witnesses, to read their proofs of evidence in full so that their case would be fully set out in public. This is not so with the opposition. Groups or individuals who appeared in person were requested to summarize their evidence, sometimes to the considerable disadvantage of the objectors, as additional personal time had to be devoted to preparing such a summary in a cogent but no less coherent form.

In addition there were 112 entirely written submissions, of which the inspectors stated on the final inquiry day (p.79 in transcript): "I would like to add that I will take into account *all* these written representation in preparing my report."

I fear that because of your editorial stance that the structure and scope of the proceedings were inappropriate to the issues that ought in your judgement to have been under review, you may have overlooked some very important aspects of changes in energy policy that undoubtedly have emerged from the inquiry itself, such as nuclear waste management. No doubt these issues will persist in new, rather less exotic forums.

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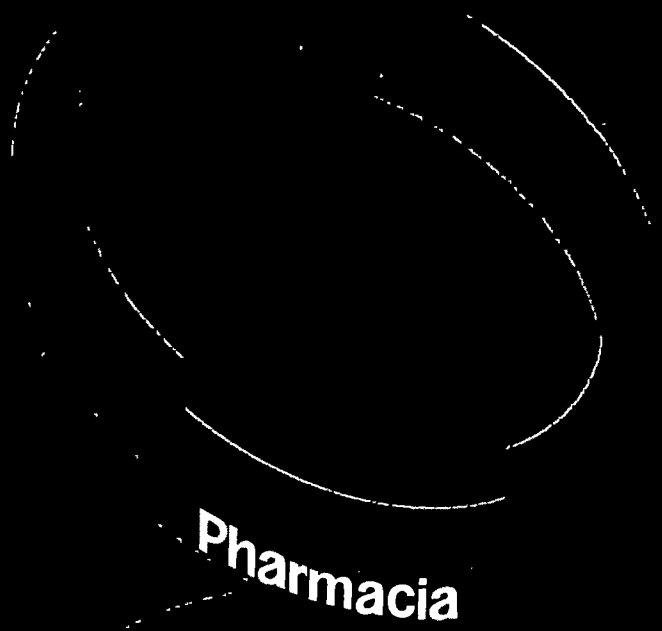
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Reader Service No.25

New ways with aggregation

An improved calculation with the classical equations of aggregation suggests new tasks for those who carry out computer simulations of these problems.

THE fashion for the study of aggregation in all its forms (from the formation of smoke particles to the polymerization of chemicals) is readily explicable. Quite apart from the several practical applications in which a better understanding would be valuable, computer simulations of aggregation processes have opened up an intriguing line of enquiry, confirming among other things that many aggregating particles do indeed have fractal, or non-integral, dimension.

General interest in aggregation is not, of course, new, but goes back at least to Smoluchowski's work in the 1920s. But how to make a bridge between the recent numerical work and the now-classical but phenomenological equations for which Smoluchowski is best known? Two people from the University of Utrecht, P.G.J. van Dongen and M.H. Ernst, have now made a brave attempt (*Phys. Rev. Lett.* **54**, 1396; 1985) which, among other things, has the virtue of suggesting where the computer people should next look.

The two approaches are as different as they could be. The computer simulations are typified by the model of T.A. Witten, which has been much elaborated since it first appeared in 1981. Particles are allowed to diffuse from the edges of some piece of lattice (square or 5 cubic, say), with instructions that they should stick to an aggregate grown from a single particle at the centre if they should happen to reach an immediately neighbouring position. Physically, the circumstances may be likened to those in which crystal growth is limited by the speed with which solute molecules can diffuse through a solution. Aggregates formed in this way do indeed have fractal dimensions. Recent elaborations of this simple model have allowed particles to shift their position after reaching the growth surface of an aggregate, or restrict occupancy of relatively inaccessible sites.

The Smoluchowski argument is more formal, and based on an enumeration of the ways in which aggregates of different sizes can stick together to form still larger aggregates. (The opposite process, disintegration, is supposed not to happen.) Aggregates containing N elementary particles will be formed (to a first approximation) only from pairs of smaller aggregates whose combined size is N. The rate at which this happens will be proportional to the number (or concentration) of each possible pair of constituent sub-aggregates, that is to the product $c_j c_k$ where j and k add up to N. The snag is that the constant of proportionality will depend on the size of each member of the pair of sub-aggregates, so that the rate of formation of particles of size N is something like $K(j,k)c_j c_k$ summed over all possible pairs of constituent sub-aggregates. (A factor of two enters if the sum is written as a sum over both indices j and k.)

The rate at which aggregates of size N disappear is similarly calculated, but now aggregation with a particle of any size will remove an aggregate from the N-sized cohort. The result of adding these two rates together algebraically is a simple-looking set of equations expressing the rate of change of the quantities c_j which are nevertheless intractable (because they involve products of the variables, and are thus non-linear). But this, of course, is irrelevant because nothing is known about the coefficients $K(j,k)$. The hope that the computer simulations might throw some light on the nature of this function is one of the reasons why the subject has recently been fashionable.

Experiment, in the nature of things, can be of only limited help. Light-scattering from an aerosol or a colloid can, for example, provide information about the mean size of aggregating particles and some information about the size distribution. But the chance of defining the infinite set of coefficients $K(j,k)$ by the results of even the most refined experiment is plainly small. So the trick is to try to pin down the coefficients K, considered as a function of two particle sizes, from what scraps of information there may be. And a few generalizations are possible. In aggregating systems, it appears, the mean size does increase steadily with time. Experiments have also shown that in many kinds of aggregating systems, the evolving size-distribution is independent of the initial distribution when a sufficiently long time has passed.

Von Dongen and Ernst set out to generalize an earlier discussion of the problem (in 1966) by S.K. Friedlander. They restrict themselves to functions K which assume the form $j^p k^q$ when the aggregates are large, which is one sign of how much more needs to be done in this field. There are reasons for supposing that the sum of the two constants p and q cannot exceed 2.

Some of the results are nevertheless simple and striking. The formation of a gel from, say, a coagulating colloid requires that the sum of p and q should be greater than unity. Indeed, it turns out that this condition is also sufficient in the sense that if the criterion is satisfied, the system will inevitably gel. And there is much that can be said about the size distribution of particles in a gelling system, both before and after they have set.

The chief interest of the new calculations will however turn on what they have to say about the scaling behaviour of the size-distribution function in all kinds of aggregating systems. Experiments as well as computer simulations suggest that the size distribution of particles in an aggregating system has a degree of uniformity in the sense that the variables c_j (which change with time) can be related to the average aggregation size (say s) by an expression of the form $s^{-r} f(j/s)$ where r is a constant and f some function of a single variable. Von Dongen and Ernst are able to calculate more about the characteristics of such a function, and of its implications, than has previously been possible.

That there should be a simplication of this kind is really remarkable. What it implies is that, with the passage of time, an aggregating system will evolve in such a way that the size-distribution of the particles is similarly related to the mean particle size. Part of what van Dongen and Ernst succeed in doing is to calculate the properties of the unchanging distribution function, in practice by calculating its moments (the mean, variance and so on).

While the results obtained in this way are not inconsistent with what has been learned from computer simulation, there is clearly a great deal to be learned about the relationship between the simulations and the mathematical form of the coagulation function. One would have thought that there is now plenty of promise in attempts to simulate the aggregation process for a few of the special circumstances in which van Dongen and Ernst have shown the equations to be reasonably tractable.

A particularly valuable goal would be the definition of the exact form of the coagulation function when most aggregates are still small. The obvious difficulty is that the numerical programs so far used in these simulations are devoid of much in the way of the physics of what happens when substantial aggregates stick together. To what extent does rearrangement then become possible, for example? That is also a challenge for experimenters. May it be one of the unplanned benefits of the interest in nuclear winter?

John Maddox

Sex chromosomes and evolution

Haldane's Rule OK

from J.S. Jones and Nick Barton

THERE are few rules in biology, and most of those which have been proposed — Cope's Law of the Unspecialized, Haeckel's Biogenetic Law and many others — have sunk into obscurity or have been so confused by exceptions as to lose much of their force. One survivor of more than sixty years is Haldane's Rule: "When in the F_1 progeny of two animal races one sex is absent, rare or sterile, that sex is the heterozygous (heterogametic) sex"[1]. From the paper of J.A. Coyne on page 736 of this issue the genetic basis of this rule can now be seen to reflect some fundamental processes in developmental biology, models of sex determination and speciation[2].

Haldane's rule applies not only in the familiar cases in which it is the males which are heterogametic — for example, the sterile XY male donkeys which result from a cross between horse and an ass — but also in those animals (such as birds, butterflies and snakes) in which the female is the heterogametic sex. Hence males are not innately more sensitive to the effects of interspecific hybridization than are females. Haldane himself (and many of his successor's) felt that the sterility of heterogametic hybrids results from an imbalance between sex chromosomes and autosomes: XX hybrids have two complete haploid sets while XY hybrids lack the full complement of chromosomes from one parental species that is necessary for fertility. Coyne, however, has now shown that, in *Drosophila* at least, this is not true, and that a different mechanism is involved.

Drosophila simulans can hybridize with *D. mauritiana* and *D. sechellia*. Making use of an attached-X stock of *D. simulans*, Coyne generates hybrid females in which both X chromosomes come from *D. simulans*, but one set of autosomes comes from each of the parental species. Such flies have the same degree of discordance between X chromosomes and autosomes as do XY hybrid males, and might be expected to be sterile. Instead, they are quite fertile. X-autosome interactions cannot explain the sterility of heterogametic hybrids: Coyne suggests that it must arise from genetic interactions between the X and Y chromosomes of the parental species.

He confirms this suggestion by producing hybrid flies which (as far as is feasible given the genetic limitations of the stocks available) differ only in their Y chromosomes. Substitution of a *D. mauritiana* Y chromosome into flies which have a *D. simulans* X chromosome leads to a 96 per cent drop in the production of motile sperm, indicating that interactions between X and Y are indeed responsible for their hybrid sterility. Similar interactions might also be responsible for the failure of reproduction in species in which females are the heterogametic sex, as inter-racial crosses of the moth *Lymantria dispar*, in which inappropriate X and Y chromosomes are combined, fail to produce females[3]. However, such interactions between sex chromosomes cannot explain all cases of hybrid sterility. Male sterility occurs in interspecific crosses between some grasshoppers which have an XO sex-determining mechanism (and hence lack a Y chromosome)[4].

It has long been thought that the sex chromosomes diverged from a pair of homologues differing only at a locus determining sex. Meiotic pairing between X and Y in man is quite extensive[5], suggesting that there is homology between the two. A 36-kilobase stretch of single-copy DNA which has been translocated to the human Y chromosome during recent hominid evolution has been shown to diverge by less than one per cent from an equivalent sequence on the X chromosome[6,7] and a 30,000 M_r protein is coded for by a gene on the tip of the human X chromosome which is also present on the Y chromosome[8].

Male sex determination in mammals demands the cooperation of both sex chromosomes[9]. The Y is necessary for testis and sperm formation: human XO individuals are phenotypically female, and in some marsupials that are mosaics for XO and XY cells, spermatogonia are always XY. There is a testis-determining locus on the mouse Y chromosome, mutants of which can lead to male sterility[10]. The X chromosome is also implicated in disorders of male sex determination. In the primary spermatocyte of normal males, the single X chromosome is inactivated. Any retention of its function interferes with fertility: XXY humans are sterile (perhaps because of the presence of an active X in spermatocytes), and translocation of part of the X to an autosome can also lead to male sterility. The Y chromosome may be involved in the repression of the X which is necessary for successful spermatogenesis[11]; this may be why XX human males and 'sex-reversed' male XX mice (both of which have undergone a translocation of part of their Y chromosome to the X) show early degeneration of germ cells[12,13]. Sterility of this kind may result from failure of the Y-linked repressor locus in its new chromosomal milieu; male mice in which the sex-reversed allele is on the Y chromosome are quite fertile.

Direct genetic evidence for the existence in *Drosophila* of Y-linked genes that suppress related loci on the X has recently emerged. The *D. melanogaster* Y carries several genes responsible for male fertility, most of which encode components of sperm[14]. Deficiencies of any of these lead to a series of errors in spermatogenesis. One such mutant is known as *stellate*. Flies bearing this allele suffer from a derangement of meiosis and the production of star-shaped crystals in the primary spermatocyte[15]. A cloned segment of DNA which hybridizes with the *stellate* locus has now been found to contain a 1,250 base-pair segment repeated at least eight times in tandem, which is present on both the X and Y chromosomes[16]. In XO individuals (which in *Drosophila*, unlike mammals, are male) the X-linked copies of this region produce up to 70 times more RNA than is produced by the *stellate* locus in normal XY males, providing direct evidence that genes on the Y are repressing others with a related function on the X, and further support for the view that male fertility involves interaction between these chromosomes.

The distribution of the *stellate* sequence shows considerable differences among various members of the *D. simulans* group. Hence the sterility of heterogametic hybrids — Haldane's rule — may arise from a failure of X-chromosome repression by a foreign Y. The same seems to be true in mice[17]. When a Y chromosome from *Mus domesticus* is transferred into a laboratory strain of *Mus musculus*, XY individuals develop as infertile females or as hermaphrodites, probably because *M. domesticus* Y chromsomes bear a testis-determining locus which cannot interact with the appropriate X-linked locus in *M. musculus*. Although an autosomal sex-determining locus may also be necessary for male fertility in these hybrid mice[18] there is a parallel with hybrid sterility in *Drosophila*.

This new evidence for the importance of the sex chromosomes in hybrid sterility raises some general questions. Why, in most groups, is the germ line more sensitive to hybridization than is the soma; and why should genes that lead to reproductive isolation so often be sex-linked? One interesting possibility might involve 'selfish' DNA, propagated through the germ line despite its deleterious effects[19]. Co-evolution between such DNA and genes that are able to suppress its replication will therefore be most intense in the germ line, which might therefore be unduly sensitive to the mobilization of selfish DNA on hybridization. Examples of selfish genes include B chromosomes in Orthoptera and, in *Drosophila*, segregation distortion, cytoplasmic sterility and the mobile genetic elements that cause hybrid dysgenesis[20-23]. These genes have their greatest effect in the germ line, and their replication may be suppressed by modifiers elsewhere in the genome. It may be more than coincidence that the *stellate* locus involves a tandem repeat, whose structure is similar to other repeated elements in the genome, and whose taxonomic distribution is similar to some of the mobile elements responsible for hybrid dysgenesis[24].

As well as the genes for hybrid sterility, a disproportionate number of interspecific

differences seem to be sex linked. Not enough information is available on protein or DNA structure to test whether X-linked structural loci show undue divergence among species[25]. However, many genes for mating preferences are on the X chromosome in *Drosophila* and some butterflies[26,27], and sex chromosome rearrangements are frequently found when related Orthoptera are compared[28]. In the Australian grasshopper *Caledia captiva*, the hybrid zone for the sex chromosomes is much narrower than are those for the autosomes; there are stronger linkage disequilibria for sex-linked characters; and there is much less introgression between races for characters borne on the X chromosome than for those on the autosomes[29]. All this, together with the marked differences between male and female viability which emerge in reciprocal backcrosses, shows that the sex chromosomes are subject to much stronger selection pressures in hybrids than are the others.

This close involvement of sex chromosomes with species differentiation might arise in several ways. If speciation involves population bottlenecks (as is often suggested), then genetic drift will act more strongly on the relatively small population of X chromosomes than on the autosomes. In addition, sex-linked differences are subject to less selection against heterozygotes and recombinants in hybrids than are those carried on autosomes. Lande's estimates[30] of the rates of chromosomal evolution in insects and mammals imply an excess of sex-linked differences which is sufficient to account for those observed in nature. In addition, genes under sexual selection (which may be a potent force in the evolution of reproductive isolation[31]) may evolve more rapidly when sex linked, and the 'hitch-hiking' effect of closely linked loci may also be more important for genes on the X chromosome[25,32].

Predictions of the mechanisms of DNA replication, of molecular mimicry by parasites and of kin selection and selfish genes can all be recognized in Haldane's early writings[33]. His discovery of the regularities which underly the sterility of hybrids seems set to become equally important in modern biology. □

1 Haldane, J B S *J Genet.* **12**, 101 (1922)
2. Coyne, J A *Nature* **314**, 736 (1985)
3 Clarke, C A & Ford, E.B *Proc R.Soc* B**206**, 381 (1982)
4 Butlin R K & Hewitt G M *Biol J Linn Soc* (in the press)
5 Chandley, A C *et al Cytogenet cell Genet.* **38**, 241 (1984).
6 Page, D *et al Proc natn Acad Sci U S A* **79**, 5352 (1982).
7 Page, D , Harper M E., Love, J , & Botstein, D *Nature* **311**, 119 (1984).
8 Goodfellow, P *et al Nature* **302**, 346 (1983)
9 Gordon, F W. & Ruddle, F H *Science* **211**, 1265 (1981)
10. Eicher, E.M & Washburn L L *J exp. Zool* **228**, 297 (1983)
11 Guellaen, G *et al Nature* **307**, 172 (1984)
12. Evans, E.P , Burtenshaw, M D. & Lindsley, D.L *Nature* **300**, 443 (1982).
13 Burgoyne, P *Nature* **307**, 109 (1984)
14 Goldstein, L S B , Hardy, R.W & Lindsay, S.L. *Proc natn Acad Sci U S A.* **79**, 7405 (1982)
15. Hardy, R W *et al Genetics* **107**, 591 (1984).
16 Livak, K J. *Genetics* **107**, 611 (1984)
17 Eicher, E M Washburn, L L , Whitney, J.B & Morrow K E *Science* **217**, 535 (1982)
18 Washburn, L L & Eicher, E M. *J exp Zool* **228**, 297 (1983)
19 Doolittle, W F. & Sapienza, C *Nature* **284**, 601 (1980)
20. Shaw, M.W *Biol. J Linn Soc* **23**, 77 (1984)
21. Hartl, D *Lect. Not Biomath* **19**, 65 (1977)
22. Ehrman, L in *Coevolution* (ed Futuyma, D J & Slatkin, M) 128 (Sinauer Press, Sunderland MA, 1983)
23 Engels, W.R. *A Rev Genet* **17** (1982)
24 Hartl, D L Hiraizum, Y in *The Genetics and Biology of Drosophila* Vol 1b (eds Ashburner, M & Novitski, E)(Academic, New York, 1976)
25 Avery, P.J. *Genet Res* **44**, 321 (1985).
26 Zouros, E. *Genetics* **97**, 703 (1981)
27 Grula, J W & Taylor, O.R *Evolution* **34**, 673 (1980)
28 Hewitt, G M *Animal Cytogenetics. Orthoptera* (Gebruder Borntraeger, Berlin 1979).
29 Moran, C *Heredity* **42**, 13 (1979).
30 Lande, R *Evolution* **33**, 234 (1079).
31 Lande, R. *Proc natn Acad Sci U S A* **78**, 3721 (1981)
32. Maynard Smith, J & Haigh, J. *Genet. Res* **23**, 23 (1974)
33. Haldane, J B.S *On Being the Right Size* (ed Maynard Smith, J) (Oxford University Press, 1985).

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Chemistry

Activating the C–H bond

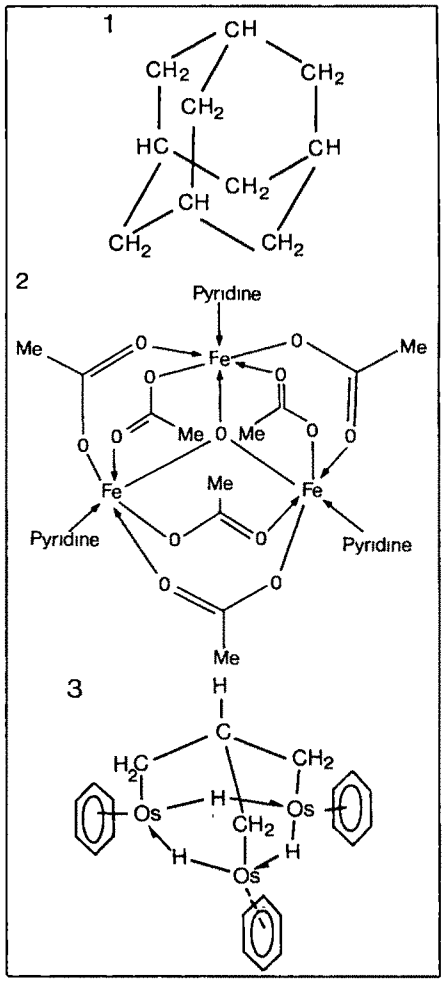
from J.M. Thomas

THE paper by Ito and Lunsford on page 721 of this issue[1] should interest chemists of all complexions, and others too, who are aroused by the challenge of effecting controlled activation of C-H bonds, especially in molecules such as methane (CH_4) the simplest of saturated hydrocarbons and the main constituent of natural gas.

It is not difficult to sever all four C-H bonds simultaneously: the ease with which this complete and relatively uncontrolled oxidation is accomplished is what makes natural gas a fuel. Ways of partially oxidizing CH_4 to produce synthetic gas ('syngas') are also well known: this is the first step in present-day industrial methods of catalytically synthesizing methanol, acetic acid and many other products, including vinyl and acetate polymers[2]. Much scope exists for exploiting syngas in new ways. The integrated process, now operated on a pilot scale by Haldor Topsøe in Copenhagen, for synthesizing gasoline using a zeolite catalyst is one example; the laboratory synthesis by Jones *et al.*[3] of acetylene using a de-intercalated graphite catalyst is another. But how far have we progressed in rivalling the comparative ease with which nature performs subtle, milder oxidation of relatively stable molecules?

For a start, organic[4] and organometallic[5-8] chemists have recently been devising ways of emulating the performance of enzymatic systems, such as the non-haem iron protein ω-hydroxylase or the iron-porphyrin-based cytochrome P-450 which selectively hydroxylate unactivated C-H bonds. And theoretical chemists have been trying to identify the factors that influence the transfer of electrons between a transition metal atom or surface and the bonding and antibonding orbitals of the C-H bond in simple paraffins[9].

In addition, at least two distinct lines of experimental attack have been used to activate C-H bonds in saturated hydrocarbons by chemical means. Organic chemists have tended to focus on molecules rich in tertiary and secondary C-H bonds, such as adamantane (1). These bonds are a little easier to activate than the primary bonds of methane. Encouraging results have been achieved using a tri-iron cluster compound (2) which, in the presence of other reagents, catalyses the conversion of adamantane to corresponding alcohols and adamantone at room temperature[4]. Like a variety of metal surfaces, bare metal atoms, under the right conditions, are undoubtedly capable of activating the C-H bond. For example, compound (3) is prepared by the co-condensation of osmium atoms and a



1:1 mixture of benzene and 2-methyl propane[8]. Its precise mode of formation is unknown, but presumably involves intermediate steps in which unsaturated metal centres of discrete osmium intermediates activate the C-H bonds of 2-methyl propane. The very existence of (3) contributes to our general understanding of how a saturated hydrocarbon may be adsorbed on to the surface of a catalyst.

In the other approach, favoured by many

physical chemists, ways have been sought of achieving the direct partial oxidation of methane to methanol, ethylene and benzene. To date, such work has entailed the use of very high temperatures, and many of the more successful experiments have used nitrous oxide as oxidizing agent, which could never figure as conveniently as O_2 or air in any commercially viable process. In that respect, the use of O_2 as oxidant, reported by Ito and Lunsford in this issue[1], is a significant advance in the general quest to oxidize methane selectively. Moreover, the catalyst they use is devoid of transition metal ions, which have been thought to be necessary constituents of efficient catalyst formulations. The mechanism of activation at the active site (believed to be a Li^+O^- pair in the lithium-doped magnesia catalyst) probably entails the formation of methyl radicals.

Gas-phase kineticists and organic chemists have, over the years, recognized the mechanistic and preparative importance of free radicals in numerous categories of reactions. But for several decades past, the role of surface-generated free radicals in heterogeneous catalytic reactions, at least in the types of reaction studied by Ito and Lunsford, has been regarded as minor. Both the preparation of solids that facilitate the formation of reactive free radicals when hydrocarbon molecules impinge on their surfaces and the role of surface-generated free radicals will doubtless receive lively future attention in formulating new strategies for the mild oxidation of methane. □

1 Ito, T & Lunsford, J N *Nature* **314**, 721 (1985)
2 Falbe, J *Phil Trans R Soc* **A300**, 205 (1981)
3 Jones, W., Schlogl, R & Thomas, J M *J chem Soc Chem Commun* 464 (1984)
4 Barton, D H R, Gastinger, M J & Motherwell, W B. *J chem Soc Chem Commun* 731 (1983)
5 Crabtree, R H, Mihelcic, J M & Quirk, J M *J Am chem Soc* **101**, 7738 (1979)
6 Watson, P L & Parshall, G W *Acct Chem Res* **18**, 51 (1985)
7 Nizova, G V, Krevor, J V Z, Kitaigorodskii, A I & Nizova, G D *Izv Akad Nauk SSSR* **12**, 2805 (1982)
8 Green, M H L & O'Hare, D *J chem Soc Chem Commun* 355 (1985)
9 Saillard, J-Y & Hoffmann, R *J Am chem Soc* **106**, 2006 (1984)

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Developmental biology

Homoeo boxes and strings for the packaging of genes?

from Brigid Hogan

NINETEEN eighty-four was a vintage year for developmental biology. The discovery of the 'homoeo box', first as a common feature of several developmental genes in *Drosophila*, and then as a highly conserved sequence in vertebrate DNA, has catalysed a lively fermentation of ideas from disparate areas of research. One outcome has been the isolation of mouse and human genes containing homoeo-box sequences. Now, on page 713 of this issue[1], Colberg-Poley *et al.* provide the first evidence that homoeo-box sequences are transcribed in mammalian cells: the fact that this transcription seems to peak transiently during the differentation of mouse embryonal carcinoma cells is bound to change some preconceived ideas about the function of mammalian homoeo-box genes, and opens up many new possibilities for experimentation.

As readers who have followed the story so far will know, the *Drosophila* homoeobox is a DNA sequence of about 180 nucleotides, discovered in at least five genes in the Antennapedia complex, three in the Bithorax complex and five outside these major gene clusters[2,3]. Mutations in specific homoeo-box containing genes can disrupt the normal pattern of segmentation either by causing a switch in segment identity (for example, from one carrying antennae to one carrying legs, in the case of *Antennapedia*) or by changing their number or anterior-posterior polarity. There is good evidence in *Drosophila* that homoeo-box sequences encode domains of proteins that are located in the nucleus and are possibly involved in DNA binding[4,5]. The general consensus, then, is that homoeo-box genes in *Drosophila* act in different combinations to select specific sets of genes defining unique properties of each of the compartments making up the larval segments. At least eight sequences closely related to the *Drosophila* homoeo box have been found in the mouse and human genome. In the case of the mouse gene described by Colberg-Poley *et al.*, there is a 95 per cent match between the amino-acid sequence that its homoeo box is predicted to encode and that of the homoeo box of the *Antennapedia* gene of *Drosophila*. Comparison of *Drosophila* and mammalian genes also points to similarities in, for example, gene clustering, 3′ intron/exon organization and the protein sequences beyond the strict confines of the homoeo box.

All this provides a strong impetus to search for a function for the mammalian homoeo-box genes. One approach is to test whether they are identical to any of the genes that, when mutated, cause mouse or human developmental defects. Already we know that one mouse gene is on chromosome 6 (the *Hox-1* locus)[6,7], and two groups[7,8] have recently assigned other homoeo-box genes to mouse chromosome 11 (*Hox-2*), and to the long arm of human chromosome 17 (*Hu-1* and *Hu-2*), near the loci for thymidine kinase, galactokinase and pro-α1 (I) collagen genes. These three genes have been mapped to chromosome 11 in the mouse (ref.8; K. Harbers and R. Jaenisch, personal communication) and it is reasonable to assume that *Hox-2* is close by. This brings within the range of speculation[8] the possibility that the gene at the *Hox-2* locus is identical to *Tail-short* (*Ts*), a gene that is lethal when mutant and homozygous, and maps close to thymidine kinase and galactokinase on mouse chromosome 11.

Fortunately for today's molecular biologists, many mouse developmental mutants have been the subject of much classical embryological and genetic study. Reference to the work of Deol[9] on the embryology of mice with one normal and one mutant *Ts* gene shows that although they develop many interesting skeletal abnormalities, early defects are also seen in non-segmented tissues, including a marked reduction in the number of blood islands in the extra-embryonic yolk sac. More recently, Patterson has described how mouse embryos that are homozygous for *Ts* die before implantation and can be dectected as abnormal even by the eight-cell stage[10]. So, if there is allelism between *Ts* and *Hox-2* (or another homoeo-box gene), at least one homoeo-box gene product is expressed at a very early stage and possibly in a wide range of tissues. Such speculation is not without foundation, as a homoeo-box gene of *Xenopus* is transcribed in the oocyte, and then again from the neurala stage at least up to the swimming tadpole[11].

Detailed studies on the transcription of homoeo-box genes in the mouse embryo will depend on good *in situ* hybridization data. Meanwhile, Colberg-Poley *et al.* have looked at transcription in F9 mouse embryonal carcinoma cells in culture. There are many tumour and embryo-derived cell lines now available, with a range of developmental potentials — from F9 cells, which hardly change at all unless treated with retinoic acid, to EK or ESC cells, which are difficult to restrain from differentiating into diverse cell types. Even when treated with retinoic acid, F9 cells give rise only to cells that express markers of extra-embryonic tissues, either visceral or parietal endoderm depending on the exact conditions[12]. In differentiating along these alternate pathways, the cells transcribe new sets of genes and switch off others. Colberg-Poley *et al.* find that a new 2.4-kilobase RNA, which hybridizes to their homeo-box probe, appears in F9 cells 17-24 h after the induction of differentiation; four days later this RNA is no longer present. The authors hint that RNA which hybridizes to the same probe is also present in normal mouse embryos, in both embryonic tissues and extra-embryonic tissues.

These results suggest that the pattern of mammalian homoeo-box gene transcript-

ion may be very complex and is not necessarily confined to specific regions of the embryo during the time when the serially repeated somite-derived tissues are being established, as might be expected by analogy with their expression in *Drosophila* embryos. Perhaps homoeo-box genes are needed for a much wider range of decision-making processes in mammalian embryos — unless, of course, they have nothing to do with development at all. For exploring these possibilities, F9 cells can be grown in large amounts so that cDNA libraries can easily be produced, and antibodies to fusion proteins used to try and isolate homoeo-box-containing proteins.

Such proteins promise to have extremely interesting properties. A possible homology between a 20-amino-acid subdomain of the homoeo box and the *MATa1* and *MAT α2* proteins of yeast, and the helix-2-turn-helix-3 region of known DNA-binding proteins such as bacteriophage λ Cro protein and *Escherichia coli* CAP protein, has been emphasized[4,13,14]. However, the rules governing prediction of three-dimensional structure from amino-acid sequence are not yet sufficiently refined for these homologies to be more than suggestive. Another motif of homoeo-box proteins is the presence of long strings of a single amino acid in regions flanking the homoeo box; repeats of glumatic acid (15 at the 3′ end of the clone described in ref. 1) glutamine, alanine and proline have been reported[1,4,15]. These strings may act as spacers between different domains of the protein designed for functions such as DNA binding, RNA binding or interaction with other regulatory proteins. Perhaps assemblies of boxes and strings act in various combinations to pack up DNA that is no longer needed, or to link new batches of active genes to the nuclear matrix. □

1 Colberg-Poley, A M , Voss, S D , Chowdhury, K & Gruss, P *Nature* **314**, 713 (1985)
2 Gehring, W J *Cell* **40**, 3 (1985)
3 Scott, M P *Trends Genet* **1**, 74 (1985)
4 Laughon, A & Scott, M P *Nature* **310**, 25 (1984)
5 Beachy, P A , Helfand, S L & Hogness, D.S *Nature* **313**, 545 (1985)
6 McGinnis, W , Hart, C P , Gehring, W J & Ruddle, F H *Cell* **38**, 675 (1984)
7 Rabin, M *et al Nature* **314**, 175 (1985)
8 Joyner, A L *et al Nature* **314**, 173 (1985)
9 Deol, M S *Proc R Soc* B155, 78 (1961)
10 Patterson, H F *J exp Zool* **211**, 247 (1980)
11 Muller, M M Carrasco, A E & De Robertis, E M *Cell* **39**, 157 (1984)
12 Hogan, B L.M , Barlow, D P & Tilly, R *Cancer Surv* **2**, 155 (1983)
13 Pabo, C O & Sauer, R T *A Rev Biochem* **53**, 293 (1984)
14 Miller, A M., MacKay, V L & Nasmyth, K A *Nature* **314**, 589 (1985)
15 Poole, S J , Kauver, L M , Drees, B & Kornberg T *Cell* **40**, 37 (1985)

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Archaeology

Megalithic recycling in Brittany

From Paul G Bahn

A REMARKABLE discovery made and recently published by C.-T. Le Roux[1] links two of the most famous megalithic monuments in Brittany — the tomb of Gavrinis, known for the superbly carved orthostats lining the passage, and the passage grave of La Table des Marchands at Locmariaquer, which also bears fine examples of megalithic art[2].

The underside of the capstone of the Table des Marchands (lower section of figure) has deep carvings of a beautiful hafted axe with two loops, a 'crook' and a couple of 'commas'. In addition, at the broken end, there are more lines which some observers have thought to be a "curious motif with a double crook"[2], whereas others saw them as part of a quadruped. Because part of this engraving is obscured by an orthostat, it has seemed likely that the slab formerly stood upright with its decoration clearly visible, but was then re-used. This hypothesis has also been made for the capstone of the tomb of Mané Rutual, which also has carving on its underside and is almost 12 m in length[3].

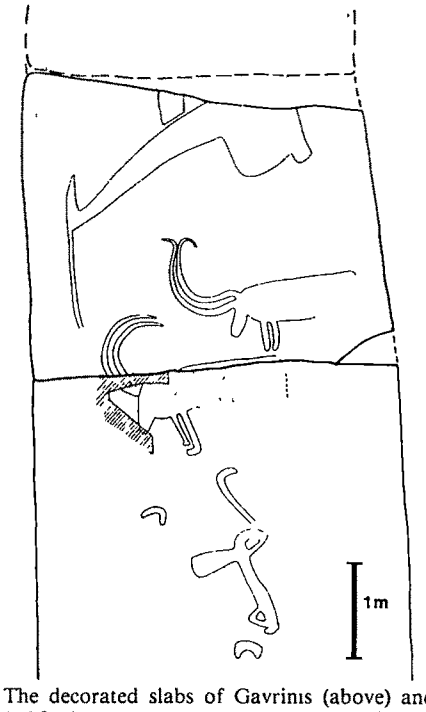
Le Roux's discovery has finally brought proof of this hypothesis. During restoration work on a capstone at the tomb of Gavrinis (now located on an island that was still linked to the mainland when these monuments were constructed), carvings were revealed on the upper side that had been invisible ever since the tomb's tumulus was set in place. The slab had clearly been dressed by man at the sides, and was broken at either end. Its carvings (central section of figure) comprised a great 'hache-charrue' (axe-plough), most of a horned animal and the horns of a second. Despite some resemblance to a mouflon or ibex, it is clear from the thick muzzle, short neck, straight back, angular rump and double-curved horns that a bovid is depicted.

The dimensions and grain of the rock, the shape of its break and the nature of its motifs all led Le Roux to link it with the Table des Marchands capstone. Sure enough, the two fragments would fit together, revealing a second bovid. Moreover, a similar rock used for a capstone in the long tumulus of 'er-Vinglé', just north of the Table des Marchands, was probably broken off from the other end of the Gavrinis slab (upper section of figure).

If so, the reconstituted stela would have stood about 14 m high, 3.7 m wide and 0.8 m thick, weighed about 100 tons and been decorated on the central portion of one face. It would have been second in size only to the Grand Menhir Brisé, 'er-Grah', which now lies in four fragments beside the Table des Marchands, and was over 20 m in length. It too was deliberately dressed and has an 'hache-charrue' carved on one of its fragments[2].

Because two of the three fragments of the 'new' stela are at Locmariaquer, it is likely that the block originally stood somewhere nearby, perhaps in the company of the Grand Menhir Brisé and the great carved endstone of the Table des Marchands. This means that the Gavrinis slab, of about 7.5 m^3 and weighing 20 tons, was transported 4 km (as the crow flies) across several water courses.

It has been suggested that the discovery implies a megalithic phase that preceded the tombs and involved great decorated stelae which, later, were deliberately thrown down and broken with wedges into fragments used in the building of tombs[4]; as the carvings were hidden to view at Gavrinis, they must have lost all significance. Le Roux is more cautious and envisages coexistence and complementarity of the menhirs and the tombs. Dating is not good enough to distinguish between the two possibilities. Gavrinis was perhaps built around 5,200-5,000 BP, and its use may have ended about 4,500 BP; but we have no idea when the stelae were put up or how long they lasted. Their decoration may provide proof of a very early use of the plough and of bovids for traction in the western-most part of Europe. But many questions about the history and function of these impressive but enigmatic monuments remain unanswered. □



The decorated slabs of Gavrinis (above) and Table des Marchands (below), as they would have fitted together (after C.-T. Le Roux).

1 Le Roux, C -T *Bull Soc Prehist fr* **81**, 240 (1984)
2 Shee Twohig, E *The Megalithic Art of Western Europe* (Clarendon, Oxford, 1981)
3 L'Helgouach, J in *Bretagne Livret-Guide de l'Excursion* (ed Giot, P -R) 129 (IXe Congr U I S P P , Nice, 1976)
4 Mohen, J -P *La Recherche* **15**, 1528 (1984)

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Astronomy

A giant black hole at the centre of a dwarf galaxy

from C Martin Gaskell

THE dwarf elliptical galaxy M32, one of the satellite galaxies of the nearby spiral galaxy M31, has recently been shown to have a central dark object with a mass five million times greater than the Sun[1]. This is the most convincing case yet of a massive black hole in the centre of a galaxy.

Signs of violent activity are to be found in the nuclei of many galaxies. They range from modest goings-on in our own Galaxy to the tremendous outpourings of energy in quasars. The source of this energy is believed to be the accretion of matter onto massive black holes[2,3] but the evidence is still only indirect. One obvious way to detect a massive black hole is through its gravitational attraction on nearby stars. In 1978, the late Peter J. Young and his collaborators[4,5] claimed to have found just such an effect; in the very centre of the giant elliptical galaxy M87, the average speed of the stars shoots up dramatically, as though by sudden strong gravitational pull. M87 is certainly a galaxy in which one would expect a massive black hole because it is relatively active, but the claim soon came under attack. Theoretical studies[6-8] showed that the observations of Young and his collaborators[4,5] could be explained without invoking the presence of a black hole (of 5×10^9 solar mass) if the orbits of the stars were predominantly radial (rather than random). Further observations[9] showed a dense concentration of stars in the centre of M87, which also had to be taken into consideration. The presence of a black hole in M87 remains possible but is certainly not necessary.

M32 has perhaps a thousand times fewer stars than a giant elliptical galaxy like M87 and is certainly not an active galaxy. It does, however, show an unexplained concentration of stars towards its centre. Using the 200-inch telescope on Mt Palomar to study the motions of the stars in and near this central concentration, John Tonry has now found that, as with M87, the dispersion in the velocities of the stars rises suddenly towards the centre. But unlike M87, the system of stars as a whole is clearly rotating in M32, so radial orbits cannot be the explanation; there has to be some excess mass there. Moreover, this mass is more than can readily be accounted for by the stars alone; an extra five million times the mass of our Sun is present, according to Tonry. A massive black hole is the most likely explanation.

If there is a black hole of such size in the middle of M32, why is the galaxy totally inactive? The answer is probably that there is nothing to fuel the black hole; gas escaping from the galaxy rather than fal ing into the centre, as it would in a mor massive galaxy.

M32 is by no means unique in having dense central condensation of stars. It ju happens to be very near and is therefo easy to study. To make comparable obse vations on more distant examples will nee better resolution than can be achieved fro the ground. When the Space Telescope (di to be launched towards the end of ne year) turns on the nuclei of these galaxi I, for one, am pretty confident that the too will show signs of massive objects lurl ing in their centres.

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1 Tonry J L *Atrophys J Lett* **283**, L27 (1984)
2 Zeldovich, Y B & Novikov, I D *Dokl Acad Nauk SSSR* **158**, 811 (1964)
3 Salpeter, E E *Astrophys J* **140**, 796 (1964)
4 Young, P.J , Westpal, J A , Kristian, J & Wilson, C P *Astrophys J* **221**, 721 (1978)
5 Sargent, W L W , Young, P J , Boksenberg, A , Shortridge, K , Lynds, C R & Hardwick, F D A *Astrophys J* , **221**, 731 (1978)
6 Duncan, M J & Wheeler, J C *Astrophys J Lett* **237**, L27 (1980)
7 Binney, J & Mamon, G A *Mon Not R astr Soc* **200**, 361 (1982)
8 Tonry, J L *Atrophys J* **266**, 58 (1983)
9 Dressler, A *Astrophys J Lett* **240**, L11 (1980)

Vision

Fireworks in the retina

from David I. Vaney

VISUAL information undergoes a sophisticated coding process in the retina, culminating in the diverse and functionally complex output of the ganglion cells to the brain. The receptive fields of the ganglion cells are shaped by the preceding interneurones, which include a set of neurones without axons, termed amacrine cells. The responses of amacrine cells are poorly documented because it is difficult to record from their small cell bodies, and the responses obtained may not reflect the local interactions between adjacent input and output synapses on the amacrine branches. In a recent series of elegant papers, however, R.H. Masland and his colleagues elucidate the functional properties of one small population of amacrines, those that contain acetylcholine[1]. Their findings suggest that the plexus formed by the branches of these cells provides the substrate for motion detection within subunits of a ganglion cell's receptive field.

In the rabbit retina, about four per cent of the amacrine cells synthesize and release acetylcholine. Their cell bodies are symmetrically distributed on both margins of the inner plexiform layer (IPL, the narrow zone containing processes of ganglion cells and presynaptic interneurones) and their terminals form two dense bands within the IPL[4,5]. These cells are of particular interest to retinal physiologists because they are implicated in the generation of direction selectivity in two types of ganglion cells[6]. Masland *et al.* have neatly side-stepped the problems of electrophysiological recording by studying the morphological characteristics and pharmacological responses of the whole population of these cells, using a sophisticated range of techniques. Although the shape and distribution of these neurones were presaged by previous studies[7-10] which used classical histological methods, this integrated approach provides definitive descriptions and fresh insight.

Masland, Mills and Hayden[1] hav shown that the acetylcholine-synthesizin amacrine cells identified by autoradio graphy are also selectively labelled *in viv* by the fluorescent dye diamidino-pheny indole (DAPI), so that the cells can b identified and mapped in a whole-mounte retina. Tauchi and Masland[2] have impale the DAPI-labelled cells with a micropipet under visual control and iontophoreticall injected the fluorescent dye Lucifer yello into the cytoplasm. This reveals a chara teristic dendritic morphology that has bee compared to a 'starburst' firework[9], an confirms the previous description by Vane using similar methods[11] (Fig.1). Th arborization ranges from 200-800 μm i diameter but is virtually planar, with th distal dendrites narrowly stratified in on of the two bands in the plexiform layer tha contain acetylcholine.

Masland, Mills and Cassidy[3] hav demonstrated that the two morphologic subtypes of cholinergic amacrines respon differently to visual stimuli; thos branching in the inner half (on the ganglio cell side) of the plexiform layer release transient burst of acetylcholine at light ON whereas those with branches in the oute half of the layer release a burst at light OF (Fig.2). This has been establishe convincingly by monitoring the distributio and release of labelled acetylcholine i response to visual stimulation *in vitr* while using a pharmacological agent know to block the transmission of ON response.

The most striking feature of the chol nergic amacrine cells is the extensive ove lap of their dendrites in the plexiform laye each point on the retina is covered by th dendritic trees of 25-70 cells of eac subtype[2,11], an order of magnitude greate than the overlap of the horizontal or gan lion cell arborizations in the cat retina[1]

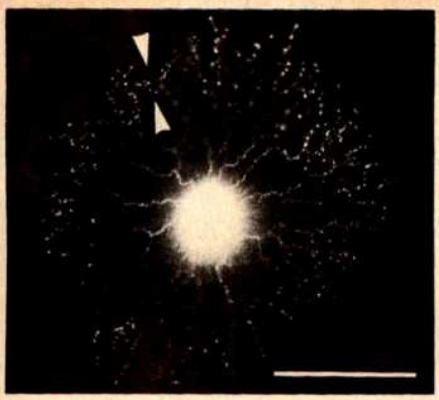


Fig. 1 Cholinergic amacrine cells in rabbit retina filled with the fluorescent dye, Lucifer yellow; the arborization in the inner plexiform layer is sharply focused beneath the blurred cell body. The characteristic appearance of these interneurones resembles a 'starburst' firework[9], an effect created by the numerous varicosities on the fine branches in the distal annular zone (between arrows). Scale, 100 μm. (Micrograph by D.I. Vaney).

Nearly all the output synapses of cholinergic amacrine cells are found on the varicose branches in the distal zone of the arborization, and make contact with ganglion cell dendrites[10]. Although the 50-80 terminal dendrites of a cholinergic amacrine are closely spaced in the plexus, a group of distal terminals may actually be separated from an adjacent group by a thin dendritic branch 200-800 μm long. Groups of terminals may thus be electrically isolated from each other and so form multiple subunits within a single arborization[12]. Tauchi and Masland[2] propose that the functional unit of cholinergic action is an aggregate of such subunits from overlapping dendritic trees. The transient local release of acetylcholine from these restricted regions of the plexus, at light ON and OFF, could provide the temporal and spatial resolution required for motion detection within the receptive field of single ganglion cells (Fig. 2).

The cholinergic plexus is so dense that there is a dendritic varicosity every few microns and, theoretically, the spatial resolution of the system could be as fine as the separation between varicosities. In practice, however, the resolution of the cholinergic plexus is limited by the less dense mosaic of presynaptic bipolar cells,

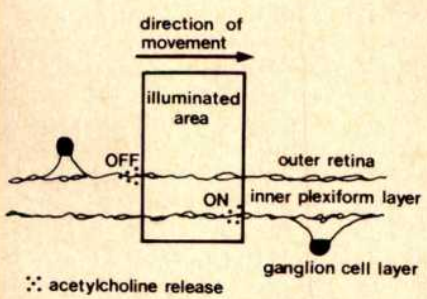


Fig. 2 Local release of acetylcholine from amacrine cell terminals in response to a moving stimulus (modified from ref.3).

which makes the overlap of branches appear curiously redundant. Moreover, dendritic replication seems a cumbersome way of producing transmitter amplification "capable of powerful local action"[2]. Such arguments assume that the cholinergic amacrines are functionally equivalent, but this need not be the case[3]: one scheme for producing direction-selective (DS) receptive fields in ganglion cells requires that the cholinergic subunits are anisotropic[6].

DS ganglion cells respond with vigorous excitation when a stimulus is moved through the receptive field in the preferred direction, whereas motion in the reverse ('null') direction inhibits them, evoking little or no response[13]. The ON-OFF DS units in the rabbit retina form four groups, each with a range of preferred directions that does not overlap the other groups. Cholinergic amacrines provide substantial direct input to DS ganglion cells and, if potentiated by the cholinergic agonist physostigmine, their excitation can overcome the null-direction inhibition[6]. It is not known, however, whether the nonlinear interactions between the excitatory and inhibitory inputs take place in the ganglion cell itself or in the presynaptic neurones. In the latter case, the release of acetylcholine would itself be directionally selective. DS ganglion cells in one group may thus receive cholinergic input from different subunits to those in the other three groups, and this would further rationalize the great overlap of the cholinergic amacrine cells.

The apparent simplicity of directional selectivity has seduced retinal physiologists for over twenty years but its mechanism still eludes them. The advances made by Masland and his colleagues provide new tools for dissecting the phenomenon and suggest that satisfaction is now within reach. □

1. Masland, R.H., Mills, J.W. & Hayden, S.A. *Proc. R. Soc.* **B233**, 79 (1984).
2. Tauchi, M. & Masland, R.H. *Proc. R. Soc.* **B223**, 101 (1984).
3. Masland, R.H., Mills, J.W. & Cassidy, C. *Proc. R. Soc.* **B223**, 121 (1984).
4. Masland, R.H. & Mills, J.W. *J. Cell Biol.* **83**, 159 (1979).
5. Hayden, S.A., Masland, R.H. & Mills, J.W. *Science* **210**, 435 (1980).
6. Ariel, M. & Daw, N.W. *J. Physiol., Lond.* **324**, 161 (1982).
7. Hughes, A. & Vaney, D.I. *J. comp. Neurol.* **189**, 169 (1980).
8. Vaney, D.I., Peichl, L. & Boycott, B.B. *J. comp. Neurol.* **199**, 373 (1981).
9. Famiglietti, E.V. *Brain Res.* **261**, 138 (1983).
10. Famiglietti, E.V. *Vision Res.* **23**, 1265 (1983).
11. Vaney, D.I. *Proc. R. Soc.* **B220**, 501 (1984).
12. Miller, R.F. & Bloomfield, S.A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3069 (1983).
13. Barlow, H.B. & Levick, W.R. *J. Physiol., Lond.* **178**, 477 (1965).

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Tectonics

Whence British Columbia?

from E. Irving

IDEAS about the origin of the western cordillera of North America are undergoing an evolution that resembles, in miniature, that which occurred in global tectonics about 30 years ago, when mobility replaced fixity as the basis for geological thought. The palaeomagnetic measurement of very large displacements among continents in the mid-1950s was followed by a decade of scepticism, until evidence from the oceans made the case for global mobility unnassailable. Similarly, measurements in the early 1950s of very large displacements in deformed belts within the confines of a modern continent met a large measure of disbelief; the orthodox view was that mountain belts were formed more or less where they are now. In the North American context, attitudes soon changed as geologists came to realize that the western cordillera was made up of a patchwork of dissimilar blocks which came together in the mid-Mesozoic (150 – 100 Myr ago) from sundry and not accurately known places. The notion of mobility therefore began to be accepted in a loose qualitative sense.

Recently, geologically young displacements have been found further and further eastward in the cordillera, especially in the Canadian sector, as if the assembled patchwork of blocks had moved northwards as a whole minicontinent during the Laramide Orogeny (80 – 50 Myr ago), when the Rockies formed. This turn of events has not been received with enthusiasm by geologists because they see little or no evidence for it in the rock record. However, it is possible that much of the direct evidence for this massive event has been obscured by deep blind thrusts, as yet unrecognized, along which large-scale horizontal transport has occurred. Rather than investigate the suggested movement of the western minicontinent, most workers have focused on easier problems concerned with its earlier assembly — with local rather than regional geology. Chamberlain and Lambert in their paper on page 707 of this issue[1] step back from the local scene and try to describe the Laramide event in its entirety.

Their scheme is based on two premises. First, the assembled minicontinent (dubbed by them 'Cordilleria', although I would have preferred the more accurate name 'Baja British Columbia' following Stone's[2] terminology) was situated much further south than at present, as indicated by palaeomagnetism[3,4]. Second, Cordilleria was driven northwards because it was attached to an oceanic plate[2], the Kula Plate[5].

Within this general kinematic framework Chamberlain and Lambert envisage two stages. To begin with, there was an interval of closure as Cordilleria moved from somewhere offshore in the Pacific Ocean towards mainland North America, a small oceanic

basin being consumed between them. Geological evidence for this lost ocean is to be found, according to Chamberlain and Lambert, in granitoid rocks formed when the oceanic crust melted as it sank beneath Cordilleria. They argue that the volcanoes that formerly surmounted these granitoid bodies were responsible for the first appearance of airborne volcanic ash in the Alberta Basin to the east about 100 Myr ago. I cannot comment on how well their proposal agrees with the geological evidence, but it does not agree with palaeomagnetism, according to which Cordilleria, at that time, was as much as 2,000 km south of Alberta[6]. Moreover, it could not have moved so early if, as Chamberlain and Lambert argue, it moved with the Kula Plate, because the latter did not form (by splitting from the Farallon Plate) until 85 Myr ago[7]. Cordilleria actually began its journey after Chamberlain and Lambert suppose it to have arrived; this does not disprove their scheme, but means only that the timing needs revision.

During the second envisaged stage, Cordilleria has reached mainland North America and procedes to slide sideways (strike-slip motion) along the Rocky Mountain Trench. It is this trench that Chamberlain and Lambert choose as the present-day boundary between Cordilleria and North America. The trench is a linear series of narrow valleys situated west of the Rocky and Mackenzie Mountains and stretching some 2,000 km from the Alaskan border, through Canada and south to the continental United States.

There is evidence of up to 1,000 km of motion along or close to the northern part of the trench[8] but across the southern trench there are similarities in Phanerozoic rocks that have usually been taken to preclude strike-slip motion. Chamberlain and Lambert regard these similarities as superficial and the consequence of wholesale Laramide thrusting across the trench, which has obscured the deeper break. In their search for this break, they point to the Proterozoic Malton gneiss complex, located astride the southern trench. They claim to have demonstrated geochemical and age differences between rocks of the complex to the east and west of the trench, and argue that the trench is a window of disrupted basement rocks exposed through overlying Laramide thrust-sheets. The earlier interpretation — that the malton gneiss 'locks' the trench, denying motion along it — is neatly turned on its head, so that it becomes evidence for motion rather than against it. Geochemists will want to see the analyses and structural geologists will wish to reassess the disposition of these remote rocky outrops, but the argument is arresting, and not lightly set aside.

I am glad that Chamberlain and Lambert have grasped this nettle. It is not difficult to pick holes in their scheme (it could hardly be otherwise with so complex a problem) but they are certainly looking at the problem in the correct way. Theirs is a useful and timely contribution which will assist discussion of the nature of the Laramide Orogeny. □

1. Chamberlain, V.E. & Lambert, R. St J. *Nature* **314**, 707 (1985).
2. Stone, D.B. *Proc. 6th A. Symp. Geol. Soc. Alaska*, 1 (1977).
3. Beck, M.E. & Noson, L. *Nature* **235**, 11 (1972).
4. Monger, J.W.H. & Irving, E. *Nature* **285**, 289 (1980).
5. Page, B.M. & Engebrestson, D.C. *Tectonics* **3**, 133 (1984).
6. Irving E., Woodsworth, G., Wynne, P.J. & A. Morrison, A. *Can. J. Earth Sci.* (in the press).
7. Woods M.T. & Davies, G.F. *Earth planet. Sci. Lett.* **38**, 161 (1982).
8. Gabrielse, H. *Bull. geol. Soc. Am.* **96**, 1 (1985).

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Genetic screening

The heredity of haemophilia

from Miranda Robertson

GENE therapy is not yet a reality. But the technology that may one day make possible the molecular repair of genetic defects is already providing a range of diagnostic and screening tools that must complicate the task of genetic counselling even as they widen the choice open to the parents of a potentially defective child. The easiest kind of genetic disease to detect by molecular means is one in which the defective gene is known and the rate of spontaneous mutation is low. This is the case for some of the hereditary anaemias, for some of which it is now possible to make gene probes that will distinguish a mutant from a normal globin gene even where the disease is due to a single point mutation. The case of haemophilia, in which the defective gene is also known and has recently been isolated and cloned, is more complicated, as a report from Gitschier *et al.* on page 738 of this issue clearly shows[1].

The commonest form of disease is haemophilia A, which affects about one individual in 10,000. The defect is in the gene encoding factor VIII, one of the cascade of plasma proteins that interact in blood clotting: haemophiliacs die from internal haemorrhage unless treated wth exogenous factor VIII. The gene for factor VIII is on the X chromosome, so usually only males are affected, females being protected by the normal gene on their second X chromosome. Females may thus be symptomless carriers: a woman whose father is haemophiliac is of necessity a carrier of the gene; one whose brother or uncle is haemophiliac may be. Treatment with factor VIII from donor plasma is effective but carries the risk of hepatitis and more recently — but very rarely — of acquired immune deficiency syndrome (AIDS); moreover about 15 per cent of haemophiliacs develop antibodies to the replacement factor VIII.

In the circumstances many female carriers would rather have an abortion than an affected child. Factor VIII, however, cannot be assayed in an unborn child until 18-20 weeks into pregnancy, and in the absence of any way of distinguishing the mutant from the innocent maternal X chromosome, some carriers have opted for early abortion of all male fetuses, accepting the 50 per cent chance that a normal child will be destroyed.

The announcement late last year that the gene for factor VIII had been isolated, characterized and cloned[2,3] opened up the possibility of precise identification of the gene defect and, with it, reliable detection of carriers and accurate prenatal diagnosis within the first 8-11 weeks of pregnancy, when abortion is safer and less traumatic. As it turns out, however, the factor VIII gene is proving less cooperative than might have been hoped.

Ideally, a mutant gene is distinguished from a normal one by a radiolabelled DNA probe that detects the mutation itself. In practice, this is really only applicable where a few identified mutations have spread through a population and probes can be tailored to each of them; where a high incidence of genetic disease is due to a high rate of spontaneous mutation, the molecular defect may be different for each affected family. This now seems to be the case for haemophilia A[4]. Moreover, as a point mutation can disable the factor VIII gene as effectively as a major deletion[4], relatively gross screening, which would only pick up a truncated gene, will miss many defects whose consequences are severe.

The alternative to direct detection of the mutation is to identify the mutant gene in individual families by means of natural sequence variations (polymorphisms) present in the population as a whole. Such polymorphisms have no effect on the function of the gene, and simply serve as markers by which one factor VIII gene can be distinguished from another. Gitschier *et al.* have discovered such a polymorphism in the factor VIII gene, and have been able to use it in carrier detection.

As a screening tool, detection of polymorphisms has several grave disadvantages by comparison with direct detection of mutations. First, because any given polymorphism may be present in perfectly normal genes as well as the mutant gene, accurate diagnosis means having enough pedigree information to establish which polymorphic variant of the gene carries the haemophilia mutation in a given family. Given adequate pedigree information, the value of a polymorphism will then depend on two more factors. The first is the number of polymorphic variants — usually there are only two; and the second is their frequency in the population at large. If there are only two variants (say, I and II) and one of them (say II) is infrequent, then a particular carrier is quite likely to be homozygous — that is, both her factor VIII genes are likely to be variant 1, although

only one of them will contain the mutation; the polymorphism will then be uninformative. (In the case of autosomal defects — where the mutation is not on the sex chromosomes — the position is further complicated by the possibility that the father may also carry the polymorphism in a normal gene; but this problem does not arise in X-linked diseases such as haemophilia because a boy inherits only one X chromosome, from his mother.)

Polymorphisms are most easily detected by bacterial enzymes (restriction enzymes) that cut DNA at specific sequences. A variation in the target sequence will prevent the enzyme from cutting at that point, and change the sizes of the fragments into which the gene is cut by the enzyme. Gitschier *et al.* experimented with 37 restriction enzymes, and found only one polymorphism in the 90,000 base pairs of the factor VIII gene that they searched[1]. This is, as they point out, remarkably low compared with, for example, globin genes. The polymorphism that they did find is revealed by the restriction enzyme *Bcl*I, and has two alleles, present at roughly 70 and 30 per cent in the population. This makes it reasonably informative: five of eight tested carriers were heterozygous.

Much more informative, however, is the polymorphism recently reported by Oberle *et al.*[5]. It lies outside the factor VIII gene itself but is in the DNA that flanks the gene and so is usually inherited with it. Termed St14, the site has 10 polymorphic variants.

The disadvantage of this marker, as with any linked marker, is that genetic recombination can occasionally separate the marker from the gene giving a false result in a screening test. Nevertheless, St14 and *Bcl*I combined with another linked marker, DX13, provide a powerful genetic screening tool in those families where the necessary pedigree data are available.

Even with routine screening, given the high rate of *de novo* mutation and the existence of a normal carrier state, haemophilia A is not going to disappear from human populations. There is much talk of using genetically manipulated bone marrow to correct genetic defects where, as in haemophilia, the site of action of the gene is the plasma. This would entail the introduction of a normal factor VIII gene into the patient's own bone-marrow cells, which do not normally express the gene. At this stage, such therapy could not be justified in the face of unknown risks, for a disease treatable by more conventional, albeit imperfect, means. Nor is it clear whether the problem of antibody production against factor VIII could be avoided, for instance by very early bone-marrow therapy. In all likelihood, we shall see.□

1. Gitschier, J., Drayna, D., Tuddenham, E.G.D, White, R.L. & Lawn, R.M. *Nature* **314**, 738 (1985).
2. Wood, W.I. *et al. Nature* **312**, 326 (1984).
3. Gitschier, J. *et al. Nature* **312**, 330 (1984).
4. Gitschier, J. *et al. Nature* (in the press).
5. Oberle, I. *et al. New Engl. J. Med.* **312**, 682 (1985).

Miranda Robertson is Biology Editor of Nature.

Mathematics

Feigenbaum's fixed function

from Ian Stewart

FOR several years there has been a flurry of activity on the topic of 'deterministic chaos' whereby quite simple systems, described by equations having no explicit random terms, nevertheless exhibit behaviour that seems to be stochastic. Using ideas from renormalization group theory (originally invented to handle problems in quantum field theory), Mitchell Feigenbaum of Cornell University was led to conjecture some very specific 'universal' properties of such systems. Feigenbaum's conjecture was proved in full rigour by M.Campanino, H. Epstein and D. Ruelle (*Commun. math. Phys.* **79**, 261; 1981 and *Topology* **21**, 125; 1982) and by Oscar Lanford (*Bull. Am. math. Soc.* **6**; 1982). Both proofs made heavy use of complicated (though exact) numerical estimates, which required computer assistance. Remarkably, Lanford — who works at the Institut des Hautes Etudes Scientifiques in Bures-sur-Yvette — has now improved the proof so that only a calculator is required.

Feigenbaum's conjecture arose from a numerical study of the behaviour of the nonlinear recurrance equation $x_{n+1} = kx_n(1-x_n)$, as the constant k increases from 0 to 4. An initially stable fixed point bifurcates into a pair of points of period 2, which in turn split into periods 4,8,16,... and so on. By the time k reaches a value around 3.7, an infinite number of these bifurcations has occurred; this is called a period-doubling cascade. Such cascades are of interest in a variety of applications, notably fluid turbulence. Feigenbaum was interested in the rate at which successive steps in the cascade developed. Let k_n be the value of k at which the nth period-doubling occurs. The ratio of gaps between consecutive bifurcation points is $\delta_n = (k_{n+1} - k_n)/(k_{n+2} - k_{n+1})$; as n tends to infinity the value of δ approaches 4.6692016.... That is, the successive bifurcation points accumulate according to a geometric progression at a rate measured by δ. On repeating the calculation for other nonlinear recurrence equations, Feigenbaum discovered that the values of δ were identical. This coincidence has important implications for experimental confirmation of mathematical models based on nonlinear recurrence equations (discrete dynamical systems), because by observing the values of k at which the first five or six period-doublings occur, one may obtain an experimental estimate for δ, to be compared with a very precise theoretical prediction.

Further investigation suggested a reason for the 'universality' of δ. If $f(x) = kx(1 - x)$ is the function being iterated in the recurrence equation, then it turns out that the second iterate $f^2(x) = f(f(x))$ has almost the same shape of graph as f, provided the axes of the graph are similarly positioned and sealed, and provided attention is confined to a suitable range of values of x. The clue to the universality lies in finding a function g for which this scaling process is exact. In other words, g must satisfy the Feigenbaum-Cvitanovic functional equation

$$g(x) = -g(g(-ax))/a$$

for a positive constant a (representing the required scale factor). The difficulty is to show that such a function g exists.

To do so, one starts with a function that satisfies this equation to a very high degree of approximation and uses it to construct a sequence of functions, by a process of renormalization, that converges to the desired solution. Abstractly one thinks of the desired g as living in a space X of functions: the renormalization operator T transforms any function h in X into a new function Th. The operator T is chosen so that the Feigenbaum-Cvitanovic equation boils down to the requirement that g be a fixed point (or fixed function) of T. Standard theorems, concerning fixed points of operators on function spaces, then finish the proof.

The difficulty is to obtain a sufficiently close initial estimate. It is here that the computer comes in. Although in the normal run of things the numbers held in a computer are subject to rounding error, it is possible to keep track of the largest size such errors can be, and hence keep exact estimates of the degree of accuracy of the calculations. In the present case, the initial choice of function h is somewhat complicated and so, therefore, are these estimates.

Lanford has succeeded in simplifying the procedure to the point at which the estimates may be performed using a hand-held calculator (here a Hewlett-Packard HP-15C, accurate to nine decimal places). The proof remains delicate, but is much simpler than its predecessors. Note, however, that to apply g to period-doubling, some additional properties must be established, which can still only be obtained using Lanford's computer-assisted proof.

The essence of the problem seems to be that the Feignenbaum fixed function g cannot be related in any very direct way to more standard functions, such as polynomials, for which methods of calculation can easily be found. Functional equations themselves have been something of a mathematical backwater. The prospect of applying them to nonlinear dynamics now makes it important to develop new and better techniques to handle their solutions. In such a way may new mathematics be born from a good physical problem. □

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Mushroom cloud the result of a meteor?

SIR — On 9 April 1984, a number of airline pilots witnessed a mushroom cloud above the northern Pacific Ocean, about 400 kilometres off the east coast of Japan. The cloud was first witnessed by the pilots of Japan Airlines flight JAL 036 at 14:06 GMT (23:06 local time) while flying route A-90 eastbound near the checkpoint PAWES. The location of the first sighting was 38.5° N, 146.0° E. The pilot reported that a large spherical cloud rose from the cloud deck below his aircraft and expanded rapidly to its full height of 60,000–70,000 feet and its full diameter of 200 miles in something between 1 min 50 s and 2 min 30 s. There was a heavy deck of clouds at the 14,000 feet level, but visibility was fair because of moonlight. About 12 s later, near 42° N, 151° E, they felt light turbulence. JAL 036 dispatched a May-day call at 14:21 GMT, put the crew on oxygen, and descended. JAL 036 was diverted to Elmendorf Air Force Base in Anchorage, Alaska, where radioactivity tests were conducted, but no trace was found. There was no communications breakup, nor any problems with the aircraft instruments.

Radioactivity tests conducted on other aircraft reporting the cloud also found no trace. Subsequent interviews with those pilots showed that the cloud exerted no fireballs or flash of lights. However, slight luminosity of the cloud was reported by several pilots. The pilot of KLM 868 reported that at first the cloud was opaque, then as it got larger it became possible to see stars through it. The phenomenon was visible for about 55 min.

The event was investigated by the US Department of Defense, Japanese Defence Agency, Federal Aviation Administration (FAA) and many others. All investigations concluded that it was not a nuclear explosion and it was possibly a natural phenomenon, but they failed to give a reasonable explanation.

We have investigated the event by querying FAA pilot interviews, newspaper reports, weather satellite photographs, and geophysical data from various institutions. Geophysical data include seismic, hydrophone, atmospheric electricity, telluric current, geomagnetic, and microbarograph data. However, none of the data we have studied contains a detectable signal from this event. Measurements of SO_2 in the ozone layer made by the National Aeronautics and Space Administration were also negative, suggesting that it was not a volcanic event.

We also obtained comment and opinions from experts in various related fields about this event. Some of the possible explanations we have pursued are:

(1) A gaint cumulus cloud.
(2) A vapour plume from the Kaitoku Seamount, 1,500 km south-west of the event.
(3) Burning of rocket fuel causing the ionization of cloud particles and the subsequent formation of a plume.
(4) A meteor causing the ionization of cloud particles and subsequent formation of a plume.

Of these possibilities, we have concluded that only the meteor plume has sufficient energy to explain the event. Meteors can enter the Earth's atmosphere at almost any angle. The speed of a meteor ranges from 15 to 110 km s^{-1}, and its height ranges from 150 km to ground level. Meteors slow down considerably upon entering the Earth's atmosphere. Normally, when a meteor does reach the ground as a meteorite, it travels the final portion of its trajectory as a dark body; it is rarely luminous below heights of 10 to 20 km.

A meteor's energy is very high. For example, the muzzle velocity of a 30-calibre bullet is about 840 m s^{-1}. A meteoroid the weight of a bullet (10 g), entering the lower atmosphere at a speed of 30 km s^{-1} has as much kinetic energy as a bullet leaving a muzzle. At this speed, even cloud particles striking at the surface of the meteor can produce a shattering effect. Collisions with atmospheric particles ionize atoms and produce visible luminosity.

Our hypothesis is that a meteor encountered the cloud deck and almost simultaneously shattered into pieces. Most of the kinetic energy was converted to heat which evaporated cloud particles, and the hot gas formed a plume. Because of the high speed of the meteor, the shattering took place over a distance spanning a few tens of kilometres, thus forming a large plume. The large distance over which the shattering occurred explains why only a slight disturbance was felt by the pilot and why microbarographs in Japan failed to detect the signal. It explains why there was neither a fireball nor a flash of light associated with the event; and it also explains the slight luminosity of the mushroom cloud.

If our explanation is correct, the pilot's experience on the night of 9 April may be the first close encounter of an aircraft with a meteor. We would welcome readers' comments on this event.

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A mechanism for prion replication

SIR — The putative causative agents of some infectious diseases which attack the brain, such as Creutzfeldt-Jakob disease of man and scrapie of sheep, have been called[1] 'prions'. Prions consist of protein, and apparently they contain no nucleic acid[1], although this contention has been challenged[2]. As reviewed recently in News and Views[3], two alternative mechanisms have been proposed for the replication of prions. (1) Activation of transcription of a host gene coding for prion protein. (2) 'Reverse translation' or protein-directed protein synthesis. There is no direct evidence for either of these mechanisms and therefore the replication of prions remains an enigma, although possible mechanisms for self-replication of proteins have been proposed[4].

We wish to propose an alternative possible mechanism for the replication of prions, which could operate if prions are indeed devoid of nucleic acid.

Prions consist of a single major protein component called[5] PrP, of relative molecular mass 27,000-30,000. We propose that PrP is a very stable slow-acting proteolytic enzyme, which may produce brain pathology precisely because of its protease activity. We further propose that the mechanism by which PrP 'replicates' is proteolytic attack of a precursor protein (which we call pre-PrP) present in many cells, and certainly in the brain, having an as yet unknown normal function. This precursor is folded in a configuration that has no proteolytic activity but, being a 'substrate' of PrP, it can be proteolytically cleaved with production of a fragment (or domain) which is PrP itself. The newly released PrP is then able to digest other pre-PrP molecules, thus bringing about multiplication of an apparent 'infective agent'. This proteolytic multiplication is reminiscent of other well-known cases in which a proteolytic enzyme (such as pepsin) acts on a precursor (such as pepsinogen) splitting off a portion to yield more of the same active protease.

The model we propose is consistent with the 'autocatalytic' time course of the diseases in question, in which there is a long incubation period followed by rapid disease progression. Moreover, the prior existence of pre-PrP in the body would account for lack of immune response (characteristically observed in these diseases), and ought to be detected also in normal cells, either directly or through its messenger.

We hope experiments will be devised to test this hypothesis, which may have relevance to other so-called 'slow infections' and degenerative diseases of the brain.

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1. Prusiner, S.B. *Science* **216**, 136-144 (1982).
2. Rohwer, R.G. *Nature* **308**, 658-662 (1984).
3. Masters, C.M. *Nature* **314**, 15-16 (1985).
4. Griffith, J.S. *Nature* **215**, 1043-1044 (1967).
5. Prusiner, S.B. *et al. Cell* **38**, 127-134 (1984).

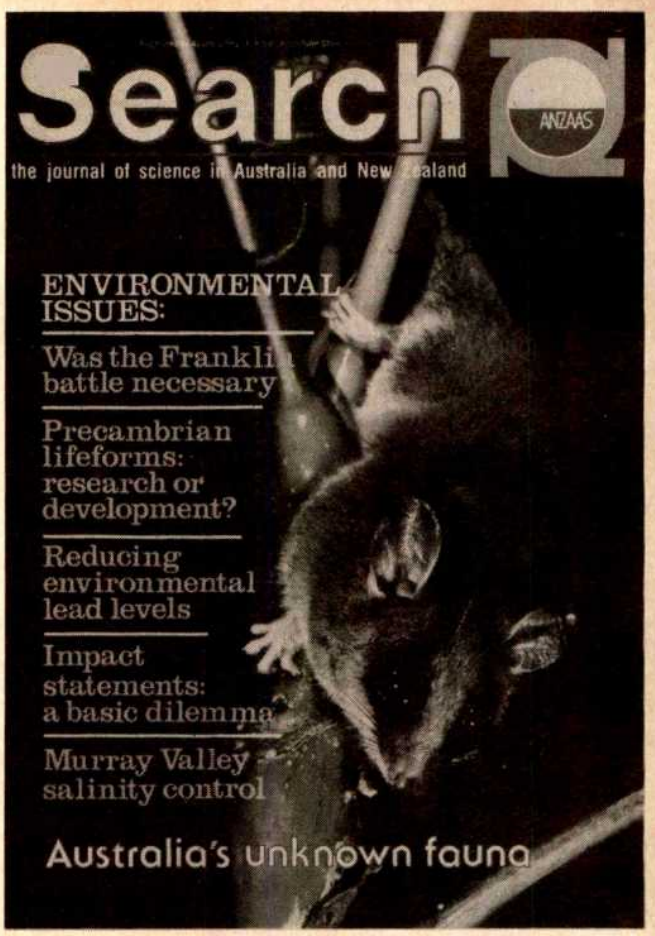
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The ragged edge

Reflections upon what is to be found in books, besides the text.

DR JOHNSON said about cucumbers that they should be well sliced, and dressed with pepper and vinegar, and then thrown out as good for nothing. This is more or less what happens when scientific information of similar insipidity is processed into books, that are catalogued and at once fade, all but a few of them, from human consciousness. To browse among the review copies that each week fill some yards of shelf space in offices of journals such as this quickly induces a desolating sense of the futility of man's endeavour. Who will actually read this tome containing the *Proceedings of the Fifth Annual Conference on Isoelectric Focussing in Polyacrylamide Gels*? Will the narrative perhaps go with more of a swing than the sinking feeling in one's viscera portends? It will not. It will be what Lewis Carroll subtitled *The Hunting of the Snark* — an agony in five fits. Why do they do it then, those battalions of authors and editors? I think it was a historian who defined research as reading two books that have never before been read in order to write a thesis that will also never be read. Perhaps Byron hit the nail on the head:

'Tis pleasant, sure, to see one's name in print;
A book's a book, although there's nothing in't.

There is material here from which more than one PhD thesis could be hewn. If there is little pleasure or improvement to be had from the texts of most books, can we find it elsewhere between the covers? There is not much fun in the index, the contribution all too often, it seems, of the author's wife. The best one can hope for is a diverting typo or two: what for instance are these congenial metabolic diseases to which this one keeps referring? And what are the particular virtues of freeze-frying? But no, there are more abundant pickings for the sociologist of science nearer the title page. The dust-flap of course often yields a morsel for the connoisseur. Here is one that comes to me at second-hand, by way of the *New Yorker*; it commends the book, a dictionary, gift bound in tooled cowhide, and notes that it is "alphabetized for easy reference". Good idea, comments the *New Yorker*. But it is more likely that you will merely be assured that you hold in your hand an integrated wide-parameter, in-depth state-of-the-art overview, that will enable the student to digest this seminal cornerstone of a rich and pregnant field; it will set new benchmark standards as a very nearly unique quantum jump, an indispensable framework, comprehensive yet concise, directed as much at the specialist research worker as at the undergraduate, and essential reading for astronomers, cytologists, palaeontologists, cytopalaeoastronomers, palaeocytologists and astropalaeontologists. But since the credit for such gems of the blurb-writer's craft cannot be laid on the author, let us start at the beginning with the epigraph. This may be in Latin or even Greek (in which the author does not have to be fluent), but if in English it should preferably be totally inscrutable. Thus: "A philosopher does not need a torch to gather glow-worms by at mid-day" — Ernest Bramah.

There follows the preface with a list of acknowledgements in which wives and family feature by reason of their fortitude in sharing for several weeks the ardours of a scribe's life. More particularly, wives without exception have typed the manuscript, compiled the index, read the proofs or supplied candid and perceptive criticism. How many such wives are wives still by the time the book is in print? The late Queenie Leavis was said to have discovered only on publication that she was not co-author of one of her husband's blockbusters. There has been a rash of lawsuits in recent years, from which emerged sorry stories of help-meets, who not only filled fountain-pens, typed manuscripts and cooked meals, but also earned money to see the husband through his PhD and were then returned to store for their pains. The scientific wife is a social species ripe for a PhD of her own.

It is customary for the author also to render thanks to a colleague or two for critical reading of the text; it would be an almost indecent breach of tradition not to conclude with the arch avowal that what errors remain are his — but the implication is that they are his friends' just the same. (As Charles II said in response to Rochester's famous mock-epitaph, "He never said a foolish thing/Nor ever did a wise one" — his words were his own, but his deeds were his ministers). There is then much citing of sources from which material has been gouged. I have only once seen a trickle of bile, such as many authors must have suppressed with difficulty; it comes from Jonathan Gathorne-Hardy's splendid treatise on the British nanny: "I must also formally acknowledge the following publishers, institutions and people for their permission, often after substantial payment, to quote from the books whose copyright they control...". The sycophantic expressions of gratitude to patrons have alas largely vanished, for the realization has grown that institutions, even those as munificent as the National Institutes of Health, have no mechanism for receiving homage or converting it into gratification. To thank the NIH has always seemed to me a little like the action of the small boy who was observed by the Reverend Sydney Smith to be stroking the shell of a tortoise in order, as he said, to please the creature; "why", said Sydney Smith, "you might as well stroke the dome of St. Paul's to please the Dean and Chapter". More entertainment was to be had from writers' relations with patrons of an earlier age. Dr Johnson's dissatisfaction with Lord Chesterfield's performance in the office is recorded in the *Dictionary*, in which he defines a patron as "one who countenances, supports or protects. Commonly a wretch who supports with insolence, and is paid with flattery". He enlarged on this in his celebrated letter to Chesterfield, in which he drew the parallel between the patron and one who observes the struggles of a drowning man and only when he reaches the shore encumbers him with help. Analogies in the support of modern science suggest themselves.

Not the least of the author's problems is the dedication. A spouse, mistress or auntie can at least be relied on to accept. (I have even seen, indeed contributed to, a multi-author volume in which the editors dedicated their collection of other men's flowers to their wife.) The most embarrassing of undesired and undesirable favours is the *Festschrift*. Some years ago an aging and eminent physicist, sensible of the approaching danger, declared in an article with the title of "Fest me no Schriften" that he did not wish to become a pretext for the unloading of unrefereed and possibly otherwise unpublishable contributions by his former students and colleagues (whom he probably did not like anyway). The Duke of Wellington once declined a dedication, explaining that he had been obliged to make a rule to do so "because, in his situation as Chancellor of the University of Oxford, he had been much exposed to authors".

As to the foreword, its object is to praise the book, and a hypocritical show of modesty can be avoided if the services of an elder of the subject can be secured. The leaden phrases that fall so easily from the pens of such illuminati are all too familiar. An ingenious ploy was contrived by Newton, who when President of the Royal Society set up a hand-picked committee to prepare a report on the rival claims of Leibniz and himself to the calculus, and then himself wrote an anonymous and fulsome foreword, commending the conclusions. But let us end with a more wholesome example, that of Boswell, who had no need of specious humility in launching his *Life of Johnson*. In what he candidly termed the "Advertisement" to the first edition, he begins: "I at last deliver to the world a work which I have long promised" and concludes, "nor will I suppress my satisfaction in the consciousness, that by recording so considerable a portion of the wisdom and wit of 'the brightest ornament of the eighteenth century', I have largely provided for the instruction and entertainment of mankind". But then his subject was better than the oxides and oxyacids of sulphur.

A pilgrim's progress

W.F. Bynum

The Correspondence of Charles Darwin, Vol.1 1821-1836.
Edited by Frederick Burkhardt and Sydney Smith.
Cambridge University Press: 1985. Pp.702. £30, $37.50.
A Calendar of the Correspondence of Charles Darwin, 1821-1882.
Edited by Frederick Burkhardt and Sydney Smith.
Garland: 1985. Pp.690. $100, £80.
Darwin Pedigrees. By R.B. Freeman.
R.B. Freeman, University College London: 1984. Pp.84. £8.

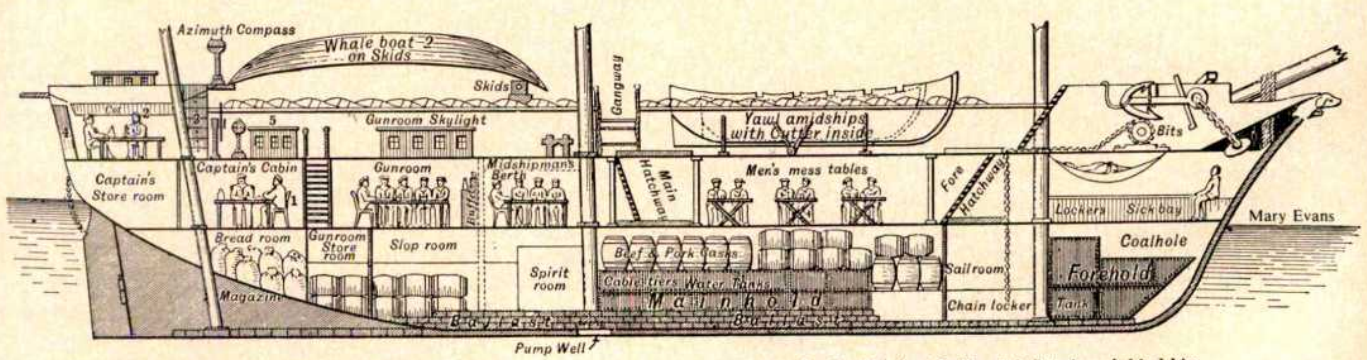
IN THESE contracting times, there is comfort in the appearance of the first volume of a set which, the publishers tell us, will take a score of volumes and most of the rest of the century to complete. The project to locate, edit and publish the complete correspondence of Charles Darwin has already been underway for a decade, supported by large grants from several American funding bodies, including the National Science Foundation, the National Endowment for the Humanities and the Andrew W. Mellon Foundation, and overseen by both British and American advisory committees. The editorial team has already involved more than 20 scholars, designated by a hierarchical variety of titles: editors, associate editors, assistant editors, research associates, research assistants and managing editor. The team has a decided Anglo-American flavour, neatly encapsulated by the two senior editors: Frederick Burkhardt, the American scholar who has masterminded the whole project, and Sydney Smith, the Cambridge zoology don who has done so much over the years to secure the magnificent Darwin archives for the Cambridge University Library. The Darwin family has always been exceptionally cooperative and generous in their dealings with Charles Darwin's Alma Mater, and it is pleasing to see the family represented on the British advisory committee through Darwin's grand-daughter, Lady Nora Barlow.

The Correspondence of Charles Darwin, then, has been, and will be, expensive to produce. Will it be worth it? After all, Francis Darwin included a number of the more important letters in his three-volume biography of his father (1887), which he followed up by *More Letters of Charles Darwin* (two volumes, 1903). Charles Darwin's daughter Henrietta Litchfield edited additional ones in her *Emma Darwin: A Century of Family Letters* (two volumes, 1905), Nora Barlow has given us the Darwin-Henslow correspondence (1967) and other scholars have published additional bits and pieces. With about 2,800 letters to and from Darwin already in print, is this present project worth the time and money? The answer is short and unqualified: yes. In the first place, 2,800 letters represent but a fraction of the 13,925 the group has already identified and calendared, and many more will undoubtedly surface.

A Calendar of the Correspondence of Charles Darwin, 1821-1882 gives us a foretaste of the pleasures in store during the coming years and will immediately prove a useful reference tool to historians working on many aspects of nineteenth-century science. The letters are listed and described, and their contents briefly summarized. They come from more than 200 institutions and private collections, and a biographical register lists over 4,000 correspondents and individuals mentioned in the series. Because Darwin lived for so many years in the relative isolation of Down House, he was probably more dependent on the postal service than any other great scientist. The output is staggering: more than 500 letters survive for 1862, almost 600 for 1874 and even in 1881, with his health visibly failing, the surviving correspondence tops 600 items. The last letter listed in the *Calendar* was written two days before he died.

Darwin, however, was not simply an ardent cultivator of the epistolary art, he was also a distinguished one. His letters are often filled with memorable phrases and shrewd insights and are a joy to read. In no future volume of the complete correspondence are the delights likely to be greater than those contained in the first. This is partly because the time span is longer, from the first surviving letter, written to the 12-year-old Darwin by Mary Congreve, to a letter of 30 December 1836 from Robert FitzRoy to Darwin, discussing publication plans for the diaries, journals and reports prepared in conjunction with the voyage of the *Beagle*. Sandwiched between are more than 300 letters to and from Darwin, and a sprinkling of letters about or affecting him (such as George Peacock's letter of August 1831 to J.S. Henslow, asking him to recommend a naturalist to accompany FitzRoy). They detail a pilgrim's progress, from a spirited young lad variously called Mr Charles, Bobby and Charley, to a mature naturalist, settled in London to work through the large collections of specimens (zoological, botanical, mineralogical and palaeontological) he had acquired on his five-year voyage around the world. The Darwin that emerges is not radically different from the one which numerous biographers and historians have described, but the assembled correspondence does present an uncluttered vision of his early personality, development, and family and social relationships. Two or three points are worth stressing particularly.

First, from the earliest years of the correspondence, Darwin was investing great quantities of libidinal energy engaging with Nature: partly in hunting and fishing, but also in collecting and studying the objects of the natural world. A long, important and largely previously unpublished series of letters between Darwin and his cousin William Darwin Fox, makes clear that insects were Darwin's first scientific passion: "I am dying by inches, from not having any body to



1. *Mr. Darwin's Seat in Captain's Cabin* 2. *Mr. Darwin's Seat in Poop Cabin with Cot slung behind him*
3. *Mr. Darwin's Chest of Drawers* 4. *Bookcase* 5. *Captain's Skylight*

Side elevation of HMS Beagle, *1832. Doubts about her size (90 feet in length, 242 tons) were a recurring feature of Darwin's letters prior to the voyage; thus (to W.D. Fox): "My objection to the vessel is its smallness.... As to its safety I hope the Admiralty are the best judges".*

talk to about my insects", he wrote in 1828; in 1831, on hearing he was off on the *Beagle*, another collecting friend Frederick Watkins wrote to Darwin, "Woe unto ye Bettles of South America, woe unto all tropical butterflies". Despite Henslow's inspiring Cambridge lectures in botany, and despite Darwin's brief geological tour of Wales with Adam Sedgwick, the Darwin who set out on the *Beagle* was first and foremost an entomologist. He very quickly became much more of a geologist, partly through the influence of Lyell's *Principles of Geology* (three volumes 1830-1833), the first volume of which FitzRoy gave him just before the *Beagle* set sail (the other volumes being sent to him in South America), but mainly through the sheer impressiveness of the geological formations and activity the voyage offered. "There is nothing like geology", he wrote home in 1834; "the pleasure of the first days partridge shooting or the first days hunting cannot be compared to finding a fine group of fossil bones, which tell their story of former times with almost a living tongue."



The Wedgwood connection — Susannah (1765–1817), daughter of Josiah Wedgwood the founder of the pottery dynasty, and Charles Darwin's mother, with her brother (centre).

Darwin's transformation from beetle collector to rounded naturalist is strikingly demonstrated in the volume. Equally significant, however, is the light it sheds on his circle: family, more distant relations and friends. The letters remind us that the sage of Down House was once a carefree young man who lay in the strawberry patches and played Housemaid and Postillion with Fanny Owen. His letters to her are lost, but Fanny's to Charles survive (mostly with strict instructions for him to burn them instantly once he has read them). One wonders what he felt when, barely a year at sea, he received his sister's letter informing him that the girl he described as "la belle Fanny", "the prettiest, plumpest, Charming personage that Shropshire possesses" was engaged to another. Fanny's letters suggest Charles was infinitely better off with Emma Wedgwood, still a shadowy figure in Volume 1. Her time will undoubtedly come in Volume 2.

This lapse into the language of romance is entirely appropriate, for these letters reflect a real world very like the one created by Jane Austen. Darwin's sisters took it in turn to write him each month, and their long and newsy letters are full of the stuff of the social history of upper middle class, provincial England during the 1830s. They send him news of the Reform Bill and the cholera epidemic, but also of engagements and marriages, births and deaths, balls and visits. His sisters despair as the voyage stretches from two to three to five years; and Darwin's own stiff upper lip begins to quiver as seasickness persists, the novelty fades and (after the visit to the Galapagos Islands), the scientific returns decrease exponentially. "In short, I am convinced it is a most ridiculous thing to go round the world, when by staying quietly, the world will go round with you." He penned this prophetic sentiment in the penultimate letter he wrote home, in July 1836.

This volume is teeming with life and exuberance. There is little of the ill health that was to plague Darwin's later years, and, because his correspondents are allowed to speak for themselves, much about his early circle, from his irascible and fascinating brother Erasmus to his quietly supportive father to his wonderfully demonstrative sisters. The letters are finely edited and presented, even if the explanatory editorial apparatus could profitably have been fuller. Several appendices provide additional information for these years, including the main events of his life, a list of books and people on board the *Beagle*, and biographical details of those with whom he corresponded or who are mentioned in the letters. The family connections can now be additionally sorted out with the aid of Richard Freeman's new book, *Darwin Pedigrees*. This reprints the scarce pedigree first produced in 1888 by H.F. Burke, and incorporates Freeman's own researches, particularly on later Darwins and on female members of the family.

For some years now, historians have referred (sometimes disparagingly) to the Darwin Industry. All it needed, it seems, was an injection of foreign capital to show itself alive, well and an immensely fruitful activity. □

W.F. Bynum is at the Wellcome Institute for the History of Medicine, London.

Happy repitition

David L. Hull

The Survival of Charles Darwin: A Biography of a Man and an Idea.
By Ronald W. Clark.
Random House/Weidenfeld & Nicolson: 1985. Pp.449. $19.95, £14.95.

BIOGRAPHIES of Charles Darwin have become as stylized and predictable as kabuki theatre — Darwin's comfortable childhood, his lazy days at Cambridge, the happy accident that put him on board the *Beagle*, his return to England after his arduous voyage, his marriage and retreat to Victorian gentility at Down House, his mysterious illness, the long gestation of the *Origin of Species*, Wallace's bolt from the blue, T.H. Huxley taking on "Soapy Sam" at the 1860 meeting of the British Association for the Advancement of Science, the eventual triumph of Darwin's theory, Darwin's later publications, his death and burial at Westminister Abbey. Clark's biography is no exception, although he does strew this familiar narrative with interesting titbits. For example, anyone reading Darwin's *Life and Letters* might notice that Darwin called his residence near the village of Downe in Kent "Down House", instead of "Downe House", and wonder why. Clark explains that the village was "Down" until 1842 when the government added the "e" to distinguish the village in England from the county in Ireland. There was, after all, the continuing Irish Problem. Darwin refused to adopt the new spelling for his own house.

Clark does not stop with Darwin's death in 1882. His book is the biography of a man, but it is also the biography of an idea. He traces the decline of evolutionary theory towards the end of the nineteenth century, its rough treatment at the hands of such Mendelians as William Bateson, the resurgence of Lamarckism, the "Monkey Trial" in Tennessee, the increasing influence of mathematics on the theory and the formation of the Modern Synthesis. He concludes with two chapters, one entitled "Molecular Biology Takes Over" and a second in which he describes recent controversies over the tempo of evolution and over cladistic analysis. These discussions are necessarily somewhat cursory, and some evolutionary biologists might protest that molecular biology has not taken over their field. The most that can be said is that molecular data have been incorporated into traditional views of the evolutionary process. With respect to the pace of evolution, Clark shows that emphasis on slow, gradual evolution has alternated periodically with more saltative views. Eldredge and Gould's punctuational model is but one more swing of the pendulum.

Another theme that recurs in the book is the social responsibility of scientists. Scientists do not publish in a social vacuum. Other experts read their work but so do theologians, reporters, high-school teachers and politicians. For example, the

anonymous reviewer of Darwin's *Descent of Man* (1872) in the London *Times* chastized Darwin for being "reckless" in publishing such "disintegrating speculations" in such perilous times. Fifty years later and an ocean away, Bateson was to be given a similar lesson when he claimed at a meeting of the American Association for the Advancement of Science that the "origin and nature of *species* remains utterly mysterious". As might be expected, newspaper headlines declared triumphantly "Darwinism Disproved".

Another 50 years on the scenario was replayed when critics of sociobiology warned that statements made by E.O. Wilson and company could easily be used by racists and



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Down House, at Downe in Kent, Darwin's home from 1842 until his death in 1882.

sexists. These criticisms in turn were taken up by Creationists as more proof that evolutionary theory is false. Even more recently, advocates of a new theory of biological classification termed "cladistics" have proclaimed that cladistic classifications are not about evolution but about patterns of character distributions, no matter how thay may have arisen. Rumours of Darwin's death in South Kensington were a result. More recently still, Tom Bethell in *Harper's* has complimented cladists for having a truly scientific outlook. "Only evolutionary agnostics like Patterson and Nelson and other cladists seem willing to live with doubt. And that, surely, is the only truly scientific outlook." As far as Bethell is concerned, the proper attitude towards evolution is the one that Huxley advocated for the existence of God — not proven. On these standards, very little has been proven in science, including relativity theory, quantum theory, not to mention continental drift. The authenticity of the Shroud of Turin is quite another matter. There is no doubting here.

Once again, Creationists are sure to make ample capital out of this intramural dispute among scientists. But what are scientists to do? No matter how judiciously they present their views, no matter how technical the journal, their statements can always be misused by those whose political or religious convictions outweigh considerations of intellectual integrity. If nothing else Clark's history of Darwinism shows that scientific disputes continue, as do marriages and divorces according to Dr Pangloss, in happy repitition. □

David L. Hull is a Professor of Philosophy at Northwestern University, Evanston, Illinois.

The conversation continued

Philip Kitcher

The Nature of Selection: Evolutionary Theory in Philosophical Focus.
By Elliott Sober.
MIT Press: 1984. Pp. 383. $33.75, £30.

VIRTUALLY no philosopher is immune to the fear of irrelevance. Despite the reassurance that one is grappling with problems that have challenged the Western intellectual tradition since the time of the Greeks, there is always the quiet voice that asks "Does anyone care?". So it is tempting to announce that philosophy is — and perhaps has always been — the servant of the sciences, and to immerse oneself in the theoretical problems that arise within some more self-confident domain of inquiry. Even this may not put an end to the whispered doubts. If philosophers turn meta-scientists, do they manage to contribute anything that a reflective practitioner could not have achieved more easily? Is philosophy only useful when it tidies the corners that talented scientists are too busy to set in order for themselves?

A long and distinguished tradition in the philosophy of science reveals the impact of combining philosophical understanding with deep knowledge of the sciences. The tradition is strongest in the philosophy of physics, where reflections on space, time, cause and chance have proven valuable both to science and to philosophy. More recently, there has been a fruitful alliance between philosophy and psychology, in which students of cognitive science have looked to philosophers for conceptual guidance, offering in return new perspectives on old problems about mind, language and knowledge. The publication of Elliott Sober's new book shows that biology's turn has now come.

On reading a draft of *The Nature of Selection* in the autumn of 1983, I formed the opinion that it would illuminate both biology and philosophy. That impression is amply confirmed by study of the published version. Sober has focused evolutionary theory for philosophers, and thereby provided new material for inquiry into the general problems of the philosophy of science, the problems of explanation, confirmation and theory structure. He has also addressed a number of major conceptual problems that currently confront practising biologists. In his hands, some disputes about natural selection are reformulated; others are dissolved.

Sober's main project is to understand the causal notions that figure in evolutionary theory. He starts from the conviction that many contemporary debates about the evolutionary process persist because the protagonists have failed to understand the character of the causal claims that they and their opponents are making. The first part of the book is devoted to an analysis of the concepts of selection, adaptation and fitness, and to a delineation of the role that these concepts — as well as the concept of chance — play in evolutionary theory. The second part develops the analysis in the context of a dispute to which Sober has made substantial contributions, the controversy about units of selection. Substantial though that previous work was, the present discussion supersedes it.

On Sober's account, evolutionary theory is best construed as a theory of forces. Evolution is a process in which typically (though not invariably) gene frequencies change along a lineage, and evolutionary theory attempts to specify the forces that produce the changes. An immediate consequence of this perspective is that the old problem about the evolutionary tautology is broached and quickly disposed of. (Those who are tempted to use the term "tautology" in discussions of evolution ought to be required to read Sober's second chapter.) A more important result is the distinction between selection *of* and selection *for*. Selection of *objects* contrasts with selection for *properties*. The distinction can be made by considering the electorate of the United States. American voters appear to select candidates who are bereft of foresight and understanding. Yet there is no selection for lack of foresight. The property selected for is surely telegenicity.

Although there are several other discussions in the first part of the book that will interest scientific readers, it is probable that they will think of the second part as where the action is. Sober's approach to the problem of units of selection starts with a chapter, allegedly designed to alienate all parties to the controversy, in which he tries to uncover confusions and fallacies in all the major positions. He continues with an analysis that makes explicit appeal to the concept of cause: a necessary condition for X-selection (group selection, genic selection, species selection and so forth) is that there should be some property of Xs that is a positive causal factor on the survival and reproduction of organisms. (Sober eventually generalizes, allowing for entities other than organisms to serve as "benchmarks" of selection.)

However, the concept of cause cannot be

● The Royal Historical Society has published a collection of correspondence to complement Jack Morrell and Arnold Thackray's *Gentlemen of Science: Early Years of the British Association for the Advancement of Science* (published in 1981 by Oxford University Press and reviewed in *Nature* **294**, 19; 1981).

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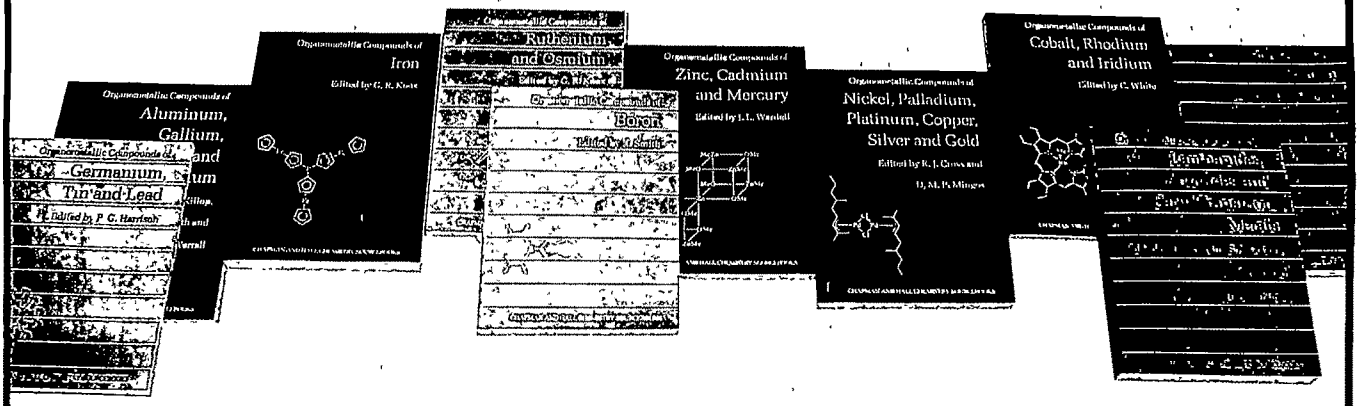
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taken for granted, and Sober invokes the philosophical literature on probabilistic causation to provide a more detailed analysis. His final suggestion is that C is a positive causal factor in the production of E in a population, if, for things in the population, the occurrence of C does not lower the probability of occurrence of E on any background of causally relevant conditions, and raises it on the background of at least one causally relevant background condition. (Roughly: smoking is a positive causal factor in the production of heart attacks if, whatever constellation of traits an individual has, smoking would make that person more prone to have a heart attack.) Sober is aware of the limitations of this analysis, which he forthrightly describes as "circular" (p.291). It would be more appropriate to treat the proposal as a recursion clause for extending our ability to identify positive causal factors. For it is this extended ability that we need to tackle the controversies about units of selection. I shall postpone the question of whether the "circularity" is bothersome.

The last chapter brings the philosophical account of causation to bear on four debates about levels of selection. Sober offers analysis of Dawkins's celebrated rowing analogy, and a diagnosis of why we should not be rushed into thinking that the gene is the one and only unit of selection. However, Sober is careful to point out that his arguments help us understand the idea of "selfish DNA" (developed by Doolittle, Sapienza and others). He also attempts to provide conceptual underpinnings for the claims about macroevolution and "species selection" that have been advanced by Stanley, Gould, Eldredge and Vrba. Here, too, is a discussion of Hamilton's ideas about inclusive fitness and local mate competition, and an extended exploration of proposals about group selection.

The account of inclusive fitness seems to me to be the least useful part of this chapter. Sober starts by suggesting that Hamilton "consolidated an insight that had been available within population genetics for some time" (p.336). Quickly adding that he does not mean that Hamilton underwrote Haldane's apocryphal quips about sacrificing himself for two brothers or eight cousins, Sober continues by reminding us that the fitness of a trait is standardly defined as the average fitness of the bearers. So Hamilton's insight can be reduced to a rather elementary idea: "There are many ways for a gene to be advantageous *on average*" (p.336).

This evaluation strikes me as parallel to the view that Darwin consolidated an insight of Malthus. Hamilton does not seem primarily interested in identifying the fitnesses of individual alleles or of allelic pairs. (That approach was later adopted by Dawkins, and is the target of much of Sober's criticism.) Inclusive fitness was introduced because of the apparent inadequacy of the classical notion of fitness for *phenotypic* traits, an inadequacy that is most obvious in traits that reduce the expected reproductive success of their bearers. When we average over the set of individuals with any such phenotype we are likely to find that the (classical) fitness lies below the mean for the population. For the lucky recipients, the kin whose reproductive success is boosted by the fact that others have the phenotypic trait in question, *are not themselves bearers of the trait*. They only enter into the set over which it is appropriate to average when we compute the chances for spreading the gene(s) that underlie the phenotypic trait, and thus calculate — as Haldane did in rough-and-ready fashion, and as Hamilton did systematically — the way in which reduced genetic representation through descendants is offset by increased genetic representation through kin.

In general, I find Sober's discussion of inclusive fitness confusing because his use of "altruist" can cover both individuals who bear genes that might (in the right circumstances or in the right combination) dispose them to sacrifice their own reproductive interests to those of others, and individuals who actually do make these sacrifices. The treatment of group selection is another story. Sober's analysis of this tricky notion is one of the high points of the book.

The distinction between group and individual selection is introduced with an apt example. We are to imagine a set of internally homogeneous populations with different average heights. We observe that tall individuals are reproductively more successful than short ones. We note also that groups of taller individuals leave more descendants than groups of shorter individuals. How do we tell if we have a case of individual selection or a case of group selection?

Everything turns on whether the causally relevant factor is the property of being tall or of being in a group with a large average height. To recognize the difference, we shall need to consider a larger ensemble of populations, asking how short individuals would have fared if they had found themselves in tall groups, and so forth. The probabilistic treatment of causality allows the conditions to be stated precisely.

Sober's work will have been done if the controversies about group selection can now be resolved by appeal to the empirical evidence. There is one obvious possible snag. The probabilistic conditions bear reference to causally relevant background conditions. If reductionists and anti-reductionists disagree on the properties that can be invoked as genuine causally relevant background conditions, then Sober's analytic apparatus may fail to bring resolution. Faced with a putative case of group selection, the reductionists produce what they are happy to count as a causally relevant property of individuals, given which the group characteristic makes no difference to the probability of survival and reproduction. If their opponents contend that the alleged property is contrived, an artefact rather than a genuine cause, then Sober's analysis will have to be extended by explicating the notion of a causally relevant background condition. So we cannot tell in advance if all the conceptual work has been done. But even pessimists should be confident that Sober has taken us a long way forward.

The Nature of Selection is not merely a book that clarifies dramatically some vexed issues. It also points to new questions and, perhaps, to refinements of causal notions that it introduces. In its achievement and in its open-endedness it deserves comparison with the work that Sober singles out as having started his own train of thought. George Williams's *Adaptation and Natural Selection* has been important to philosophers as well as to biologists for its combination of biological insight and philosophical acumen. Sober's is the answering philosophical voice, the voice of a first-rate philosopher and a knowledgeable student of contemporary evolutionary theory. His book, like Williams's, merits broad attention among both communities. It should also inspire others to continue the conversation. □

Philip Kitcher is Professor of Philosophy at the University of Minnesota. His latest book, Vaulting Ambition: Sociobiology and the Quest for Human Nature, *will be published in September.*

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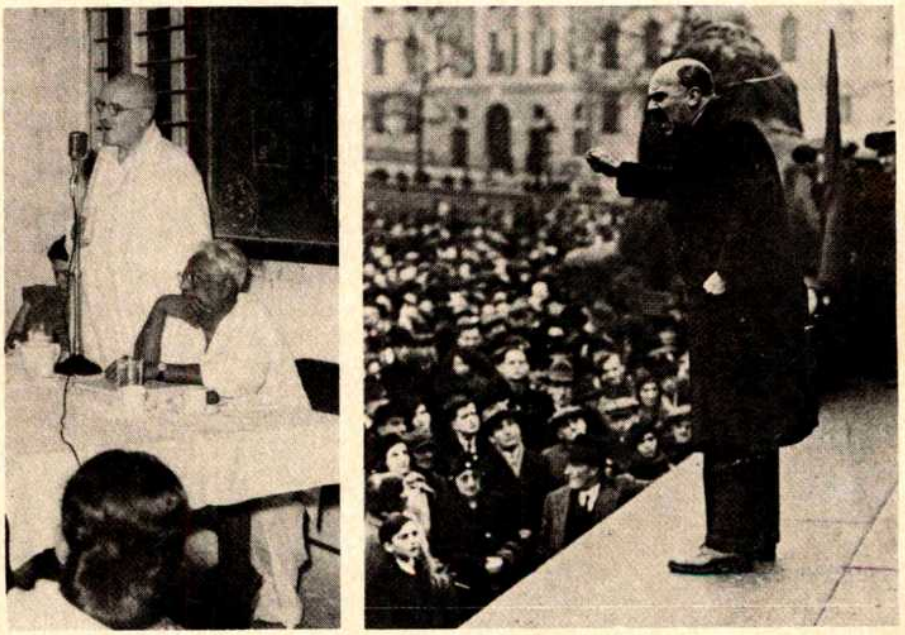
On Being the Right Size and Other Essays.
By J.B.S. Haldane. Edited by John Maynard Smith.
Oxford University Press: 1985. Pp. 187. Pbk £4.95, $6.95.

I MUST confess that when I first met J.B.S Haldane I thought him a bit of a humbug. Dressed in his *dhoti*, smoking a twisted black Burmese cigar and addressing a group of admirers in a Dutch café, he seemed unreal. Nor was I alone in that opinion. Once in an American department store where we had gone to buy some things for Helen Spurway, Haldane pulled out an Indian rupee note, announcing to the sales clerk "I'm a Hindu, you know". As he left, she turned to me and said "That's funny. I could have sworn he was one of those high-class Englishmen".

But, of course, Haldane was vastly more than a showman. He was the most incisive and wide-ranging intellect I have ever known, original, iconoclastic, a master at making connections between apparently unrelated phenomena, and by far the best popular science writer who has ever addressed an English-speaking readership. All these aspects are displayed in this collection of his essays, chosen with great judgement by his former student and colleague John Maynard Smith, essays ranging from the origin of life and the gods, to the Last Judgement, somewhere around the year 36,000,000. In between Haldane explains in plain English what "hot" means, how the organism is produced by interaction of its genes and its developmental environment, why one can survive for hours shut up in an air-tight box and how capitalism has made a commodity out of health. Written as clearly as they are, these essays tempt the reader to take them all at one sitting, but I advise against it. The best prescription is one a day followed by deep thought.

Haldane the humbug shows up occasionally, as when he quotes extensively from the Norse epic *Volospa* in the original, a bit of flummery that can only be meant to give an impression of fluency in an esoteric language. Yet it seems so unnecessary. Haldane was a person of genuinely wide learning, as shown, for example, in the essay "Is History a Fraud", where a variety of historical detail is displayed in the service of a central idea: the superiority of the practical over the merely intellectual. It is a sign of the contradiction that marked Haldane's world view that he could prefer Pasteur above Darwin because the former solved a "practical problem of living", yet at another time could praise an idea as "interesting, even if not true".

Haldane the critical scientist comes out best in "The Biology of Inequality" which shows a subtlety of understanding of the relationship between gene, environment and organism that is, even today, absent from the thinking of most biologists. Yet here again is a contradiction. Haldane makes clear in this essay that it is not the phenotype, but the pattern of reaction to a contingent environment that is inherited. Yet earlier, in "The Future of Biology", he writes of "the main gene that determined his father's mathematical powers". Nor was it simply wisdom acquired in the ten years between these essays. To the end of his life, Haldane remained committed to a hereditarianism in regard to human psychic development, a biological determinism that may seem strange in



Public faces — Haldane addressing a "United Front" meeting in Trafalgar Square, 1937 (right), and at the Indian Statistical Institute, Calcutta. The pictures are reproduced from J.B.S., *Ronald Clark's biography which was re-issued in paperback last year by Oxford University Press.*

someone who claimed to be a revolutionary socialist. The key to this apparent paradox can be found in what appears to be Haldane's preoccupation with religion.

In essay after essay in this collection Haldane returns to the subject of religion. It is not simply that the editor has chosen essays on religious subjects, such as "When I am Dead", "God-makers" and "Science and Theology as Art Forms". Haldane brings in Christianity, Hinduism, religious myth in general and an Egyptian fakir in particular as integral to the points he is making about history and science. To understand why this is so, we must realize that Haldane was born in the last years of Victoria's reign and his intellect was formed in the Edwardian period. The primary intellectual struggle in his view, a view shared by Marxists of the 1930s, was the struggle between materialism and philosophical idealism. Marxism for Haldane, as for H.J. Muller, meant the fight to overturn the superstition, both scientific and social, that marked nineteenth century ideology, and its replacement by a materialist, scientific society. Part of that materialism was a belief that biological technology including eugenic measures could be instrumental in putting society on a better basis. The view seems naive to us now. If biology is to be relevant to reorganizing society, it must be the biology of what is in our heads, not what is in our gonads. Nevertheless, we must side with Haldane's desire to replace mystifying social myths by rational realities. □

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Schools of thought

Struther Arnott

Graduate Research: A Guide for Students in the Sciences.
By Robert V. Smith.
ISI Press, 3501 Market Street, Philadelphia: 1984. Pp.182. Hbk $21.95 (North America), $24.95 (elsewhere); pbk $14.95 (North America), $17.95 (elsewhere).

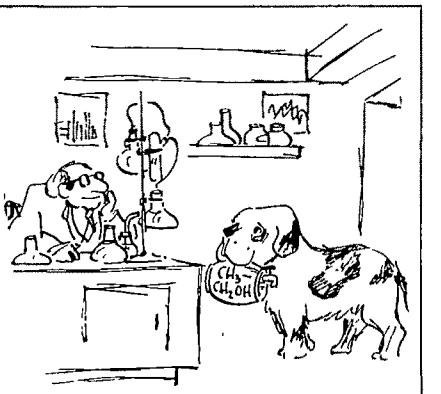
HERE is a programme instructing the ambitious mediocracy how to apprentice themselves to the trade of being a (US) scientific worker. It begins at the same inspirational level as it continues with the thought that "it is crucial for graduate students in the sciences to master this skill [in research] *because their advanced degrees depend upon it*". It concludes with the optimistic and self-congratulatory assurance that "you should expect to find a good position".

This is a "how to" manual with a vengeance: how to get started; how to choose a research field and a mentor; how to manage time and libraries; how to write dissertations and papers; how to get grant support and a job; even how to note rather than to take cognizance of and how to prepare a consent form for research on human subjects. Thank goodness the chapters on ethics and creativity are not "how to" ones. But there we are regaled with a re-run of Kekulé's reverie which produced the structure of benzene. Inexplicably absent is the crucial part of the dream where some of the dancing chains of atoms joined ends to form rings. Such persistence in missing the point is a general characteristic of this book.

There is a tidy-minded part of me which was impressed by Dr Smith's thoroughness in mustering many useful thoughts about our graduate schools. I and my colleagues surely would want our doctoral students, potential and actual, to be aware of the management structures in our (usually very large) universities, to be familiar with the various opportunities for financial support, and to be chary of over-involvement in teaching and in passive attendance at courses and seminars. However, not all the wise saws and modern instances are as pleasing. Few of us would dispute that "excessive television is a deterrent to purposeful observation" but would any of us want to be saddled with protégés needing such banal advice or with disciples who would formulate the hypothesis that "sensory deprivation in human subjects is associated with increased alienation and manifested as loneliness" only after a survey comparing deaf and blind widows with widows having normal hearing and sight?

The author does assert at one point that "research... requires certain personal traits and practices. Some characteristics must already be part of the individual, some can be improved with practice". I agree completely. What strains my credulity is the implication that a beginning graduate student typically could have postponed until reading this book the acquisition and development of the traits of a successful scholar or researcher. Yet expectation of perpetual opportunity for educational rehabilitation has been a pervasive ingredient of public policy in the United States for at least a generation. Only recently has there been general recognition of the consequences, such as high schools staffed with inadequately rewarded — and therefore indifferent or demoralized teachers — graduating, not very discriminatingly, 75 per cent of their entering classes. Better paid college professors can thereafter, much more expensively, try to remedy ingrained habits of intellectual indolence. Professors condemned to be ushers do not resist this marketplace philosophy nor strive to maintain system in national educational arrangements. The result is loss of rigour, in secondary and in higher education, and buffeting of both by fads and fashions. My own institution recently suffered from a neap tide of interest in computer science which briefly was the proclaimed major subject of more than half the freshman class in our School of Science. Today, that tide is receding, exposing a floundering population dismayed by its lack of quantitative training, analytical skills and capacity for sustained hard work.

A committee of the American Association of Colleges now has demanded baccalaureate programmes which would ensure that their alumni would reason well, would read, write and speak with distinction,



Reproduced from Science Goes to the Dogs, *a new collection of cartoons by Sidney Harris published by ISI Press. Price of the book is $7.95.*

would understand numerical data and the scientific method, and would have historical consciousness and knowledge of the language of the fine arts. Graduate schools, anxious for distinction, will demand at least this, as well as evidence of ability to study in depth which, as the AAC report describes, requires sequential learning, building on blocks of knowledge that lead to more sophisticated understanding and encourage leaps of the imagination and efforts at synthesis.

Now indeed may be the time for us to attend to the quality and configuration of our graduate schools. The main places of employment for holders of the PhD degree are colleges and universities. Population trends will ensure a 15 per cent fall in the traditional university student population by 1995. Scepticism about the value of some academic qualifications could accelerate this decline substantially. A shift in public policy towards investment in earlier stages of education could make the descent precipitous. Even without a revolution in public policy, there may be less than 100,000 academic positions to be filled in the next decade. Success will fall to those graduate schools which order their requirements and incentives so as to be able to recruit more discriminatingly. In such an environment, Dr Smith's book would be useful only to foreigners curious about some of the minutiae of the academic research environment in the United States.

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Angles on knowledge

David Miller

The Limits of Science.
By Nicholas Rescher.
University of California Press: 1985. Pp.225. $34.50, £31.50.

THE question of whether there are any limits to empirical science is one that, since Kant anyway, has never been far from the centre of the theory of human knowledge. Logical positivists persuaded themselves (and others) that meaningful investigation is restricted to the results of observation, experiment and induction. Less extreme sufferers from scientism have held that there is no knowledge but scientific knowledge. On the other side it has been suggested that the traditionally conceived method of science as one of "rational triangulation from the empirical data" (as Rescher describes it on p.198) sets more of an external limit to science than an internal one.

In this work, much of which is collated from his innumerable previous books, Rescher approaches the issue from a number of angles: whether there are topics wholly beyond the mandate of science ("no" in Chapter 7, "yes" in Chapter 12); whether science could be perfected, even in principle ("no" in Chapter 9); whether there are problems within its domain that science is in principle unable to answer ("no" in Chapters 4 and 8); whether there are significant questions that for practical (technological) reasons science cannot handle ("yes" in Chapter 10); whether science progresses steadily towards theoretical perfection ("no" in Chapter 5); whether it progresses at an instrumental level ("yes" in Chapter 6); whether extraterrestrial science might be scientifically more advanced than ours (question dismissed in Chapter 11).

The discussion throughout is thoroughly unsatisfactory: superficial, confused, philosophically unsophisticated, repetitive, neologistic, above all deeply antipathetic to theoretical speculation. For example, in the account in Chapter 11 of how our science and alien science might be related, a discussion whose only real conclusion is that there is nothing of interest to say, we read:

> A tiny creature living its brief life span within a maple leaf could never recognize that such leaves are deciduous . Science is limited to the confines of discernibility: as Kant maintained, the limits of our experience set limits to our science [p.197].

So much for the power of ideas. Yet in Chapter 8 Rescher seems properly to appreciate that science is not confined to what we can experience but a method of going wildly beyond it. In a similarly glum mood Rescher seems to suppose that because other planets might be physically very different from ours, scientists there might use

> mathematics very unlike ours . their 'geometry' could be something rather strange, largely topological, say, and geared to flexible structures rather than fixed sizes or shapes [p.177]

Just like some of our mathematics by the sound of it.

Can current science tell us anything about future science, about extraterrestrial science, about possible limits to scientific inquiry? The answer seems obviously that it might be able to, but that what it tells us might be wrong. Rescher persistently muddies this issue, gliding from the platitude that "no adequate justification can be found for the view that science has barriers" (p.130) to the assertion that it has no barriers. He seems not to have appreciated that if "present science cannot speak for future science , [if] there can be no basis for claims of inherent unanswerability" (p.129), then present science cannot speak for present science either, and there can be no basis for its assertions about the natural world. Yet Rescher is unwavering in his belief that the results of science can be justified. We are told that the advance of science amounts to obtaining "a firmer warrant for our claims", not "more of the truth"; and we are warned of the "fallacy . that moves from a picture-of-nature's being a *better-warranted picture* to its being a *better picture*" (pp.73-74). Only someone who confuses justification with truth, and thinks that all moves have to be justified, could see this move as a fallacy rather than as an attempt to discover the truth.

The question of the existence of insolubilia is itself eventually resolved in Chapter 8 as follows:

> How could we possibly establish that a question *Q* will continue to be *raisable and unanswerable* in every future state of science . ? [If we] argue that the answer to *Q* lies 'in principle' beyond the reach of science, it is difficult to see how we could maintain it to be an authentic scientific question [p.128].

In other words, science has no limits except where it has limits.

Most of the topics treated in this book are treated more than once, as though the different chapters had originated from different authors. The writing is always verbose, and horrible hyphenated words ("issue-killer" on p.17, "question-disallowing" on p.22, "hole-internal" on p.131, for example) abound. On p.52 the same 130-word quotation appears twice: in the text and in a footnote appended to it. The word-processor has a good deal to answer for. In most other respects the book is well produced. □

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• P.B. Medawar's *The Limits of Science*, first published in the United States by Harper & Row, and recently in Britain by Oxford University Press, was reviewed in *Nature* **312**, 203 (1984).

Word and number meta-magic

David E.H. Jones

Metamagical Themas: Questing for the Essence of Mind and Pattern.
By Douglas R. Hofstadter.
Basic Books: 1985. Pp.852. $24.95. To be published in Britain later this year by Viking.

DOUGLAS Hofstadter is widely and justly celebrated for his book *Godel, Escher, Bach*, and for his co-production with Daniel Dennett of *The Mind's I*. Between June 1981 and September 1983 he succeeded Martin Gardner in running *Scientific American's* "Mathematical Games" department. He gave his contributions the anagrammatical title of "Metamagical Themas". All 26 of these essays are reprinted in this book. Each now has a postscript of subsequent reflections and developments; in addition there are seven new, specially-written "Themas".

Hofstadter claims that his Themas form a scatter of random dots around his intellectual "home territory", which is concerned largely with thinking, thinking about thinking, and thinking about language. There are Themas on self-referring and self-replicating sentences, and ones on the search for consistent patterns and styles in music and in geometrical artforms. Hofstadter's own research into artificial-intelligence programming is reflected in several Themas, such as those on the pattern-recognition of sameness underlying difference, in particular the recognizable sameness of letters of the alphabet throughout unimaginably many variations of form and fount.

Other Themas continue the tradition of "Mathematical Games", dealing with such topics as Rubik's cube, the mathematics of chaos, computer languages, psychological games with numbers, and logical dilemmas and paradoxes. A rather special cluster of Themas locates a societal region in Hofstadter's territory: his concern over mass gullibility, innumeracy and some political issues. Each Thema is complete in its own right, but most are buttressed and extended by others elsewhere in the book. It's a format ideal for browsing; rather like his previous books, in fact. There is an excellent bibliography, with short notes on the works cited.

To illustrate Hofstadter's style and matter, I'll outline one Thema in greater detail, No.23 "On the Seeming Paradox of Mechanizing Creativity". Could a computer be creative in the human sense? Or must it always be, in effect, a huge fast-scanned dictionary of clichés? After considering the background to this question, Hofstadter introduces an example of

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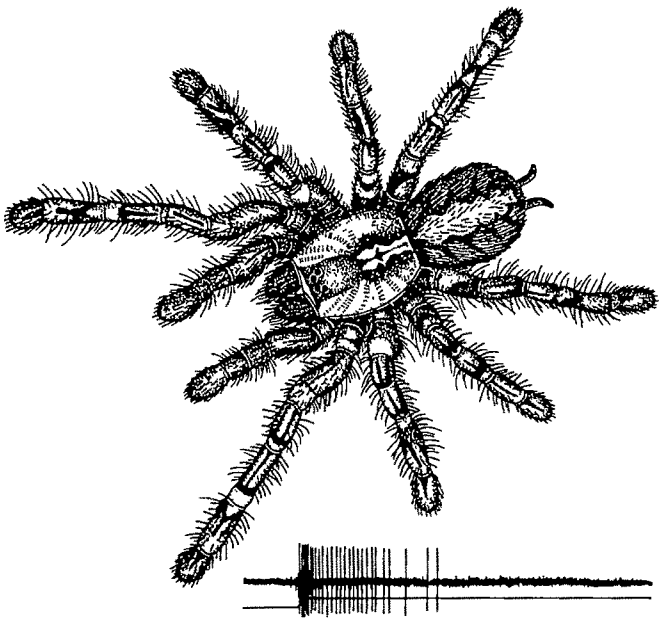
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K. Reise

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Editors: **E. Kuwert, C. Merieux, H. Koprowski, K. Bögel**

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extreme uncreativity — the blind repetitiveness of instinctive behaviour shown by the wasp *Sphex*. Typically, he gives this behaviour a name: sphexishness. Its opposite, mental flexibility, he calls anti-sphexishness; and to illustrate the notion he positions various machine, animal and human behaviours along a continuum connecting these extremes. The essence of anti-sphexishness, says Hofstadter, is the ability to break out of loops and ruts of all



Hofstadter — love of word-play.

kinds. He has a word for this, too: "jootsing" (jumping out of the system). It depends, he argues, on watching what you're doing, so as to break up any routine which is getting repetitive. This leads into his favourite topic of self-watching and self-referencing systems, recursive theorems and computer-routines, and the use of recursiveness in "creative" computer programs. He ultimately concludes that some sort of Gödel theorem forbids any exhaustive formalization of creative ability. Any conceivable creative genius or artificially intelligent program must ultimately remain a prisoner of its own style — just like the humble *Sphex*.

In the postscript to this Thema, Hofstadter throws out some intriguing remarks about the subtleties of "style" (What makes a tune catchy? Why do tradenames such as "Day-glo" and "Turbomatic" now seem vaguely dated?), and comments on a new rival artificial-intelligence strategy inspired not by recursion but by the ideas of statistical mechanics. The reader is directed to related discussions in other Themas.

Hofstadter is no mere armchair theorist. He constantly supports his ideas by appeals to practical experience or to intriguing psychological experiments. Expounding the Turing test for computer intelligence (can you distinguish the computer from a human being by its responses to questions?), he presents a transcript of an actual Turing test, with a delightful surprise ending. His discussion of the "Prisoner's Dilemma" paradox, a sort of mathematical microcosm of the tension between competition and cooperation, is enlivened by accounts of several experimental studies. Two of them were tests caried out by Hofstadter himself on human subjects, the other two were tournaments between computer programs. The tournaments marvellously illuminate the biological concept of the "evolutionarily stable strategy" — the optimum behavioural strategy towards which a group of competing organisms will evolve over the generations. This is used to suggest how socially cooperative creatures could have evolved from a primitive population of warring egotists.

Hofstadter's style of writing has its own pleasures and pleasantries. He loves playing with words; puns, anagrams and literary jokes abound. This almost dangerous infatuation with word-play at times works against him. Three whole chapters are devoted to self-referencing sentences, a self-indulgence quite unjustified by the limited conclusions reached. Another chapter presents numerous examples of literary nonsense, again without any moral that I can see. Set against the commentaries by (for example) Aldous Huxley, George Orwell and John Updike on nonsense-verse considered as a penetrating subversion of the stifling world of convention, this is very thin gruel.

The most serious lapses, though, are political. Hofstadter declares himself a natural activist, easily "fired up" by various good causes. The ones he espouses here, those of nuclear disarmament and feminism, are badly served by his simplistic enthusiasm. His anti-nuclear Thema is a sadly flat and contrived parable, arguing that if enough people devote enough time to denouncing the arms-race, it will go away. Once world leaders realize the complete symmetry of the U.S./S.U. confrontation (there's that word-play again) all will surely be well. His feminist Themas point out that in normal grammatical English usage, "he" can include "she" and so on. He seeks to make this a matter for resentment among women, and guilt in men, by means of an extraordinary attempted mapping from a region of very high guilt-density, that of race relations. Orwell, in his celebrated essay "Notes on Nationalism", remarks that the political bigot's great sensitivity to specific words and phrases probably has overtones of word-magic — the belief that the world can be changed by proper incantation. Despite his fascination with words, Hofstadter is too rational a soul to be a bigot. He admits that he may be over-acting. Certainly all previous attempts to change society by word-manipulation have merely enriched the language's glittering treasure-house of euphemisms and polite evasions.

But this is to carp. Most of Hofstadter's Themas are concerned with his areas of special knowledge and insight, and sparkle with linguistic fun, provocative insights and intellectual challenges. This random walk through his intellectual "home territory" is highly recommended. □

David E.H. Jones is a guest staff member of the Physical Chemistry Department, University of Newcastle upon Tyne, and a science consultant to industry and the media.

Life with a lighter touch

Martin Gardner

"Surely You're Joking, Mr. Feynman!": Adventures of a Curious Character.
By Richard P. Feynman with Ralph Leighton.
W. W. Norton: 1985. Pp.350. $15.95. To be published in Britain later this year by W. W. Norton.

"THE stories Feynman tells about himself would make a book", an interviewer wrote in 1963. Here, 22 years later, is that book. It is not an autobiography. You will learn nothing from it about the work on quantum mechanics that earned Richard Feynman a Nobel prize in 1965. What you will learn is a great deal about the flamboyant personality of a great theoretical physicist: his amazing range of interests, his unusual hobbies, his enthusiasms and animadversions, his dislike of pompous fools, his unpredictable behaviour and, above all, his fondness for outrageous comedy.

Feynman's famous three-volume *Lectures on Physics*, and his marvellous little book *The Character of Physical Law*, were based on taped lectures. *Surely You're Joking, Mr. Feynman!* is a taping of Feynman's uninhibited conversations over the years with his friend Ralph Leighton. "That one person could have so many wonderfully crazy things happen to him in one lifetime is sometimes hard to believe", Leighton writes in his preface.

The reason they happened is that Feynman has a knack of creating his own adventures. When, for instance, he was a young man at Los Alamos, working on the top secret bomb, he discovered a hole in the perimeter fence. Anyone else would have reported this to the authorities and that would have been the end of it, but Feynman is incapable of passing up a chance for a lark. Out of the main gate he goes, back through the hole, then out again. He keeps this up until the sergeant at the gate suddenly realizes that some strange character is always going out but never coming in.

Feynman's expert ability to pick locks followed naturally from his lifelong passion for puzzles. To demonstrate how loose security was at Los Alamos, he would secretly open the safes of top scientists, leaving little notes that said such things as "I borrowed document LA4312 — Feynman the safecracker". But such idiosyncracies were not appreciated by everyone, least of all the military. When three psychiatrists tried to test Feynman for Army service they quickly found themselves being tested. "How much do you value life?", one of them asked. "Sixty-four", Feynman answered. The Army's final verdict: mentally deficient. (The chapter on this banter, headed

"Uncle Sam Doesn't Want *You*!", is one of the book's funniest.)

My favourite anecdote, however — it's vintage Feynman — concerns a joke his friends once tried to play on him. In Japan Feynman had learned some Japanese, and in Portugal he gave lectures in Portuguese. At a party someone thought it would be amusing to see how this "man of a thousand tongues" would react if a Caucasian lady, who grew up in China, greeted him in Chinese. "Ai, choong, ngong jia!" she said with a bow. Taken aback, Feynman swiftly decided the best thing to do was imitate the sounds she made. "Ah, ching, jong jien!" he replied, returning the bow. "Oh, my God!" the lady exclaimed. "I knew this would happen. I speak Mandarin and he speaks Cantonese!".

From childhood on, anything mysterious or puzzling instantly aroused Feynman's curiosity. Growing up in Long Island, New York, he taught himself how radios work and he became the neighbourhood's youngest radio repair man. He invented ingenious experiments with ants to work out how their brains were programmed. Intrigued by hypnotism, he allowed himself to be hypnotized. To experience out-of-body hallucinations, he floated in a sensory deprivation tank. Always, wherever he was or whatever he did, the wheels in Feynman's brain never stopped whirring. Every experience posed new challenging questions. What goes on here? Why does this work? Can it be done better? The best example echoes Newton and the apple (no, that story isn't a myth). Feynman once watched someone toss a plate in the air and noticed that its wobble went around faster than the plate. It started a train of thought that eventually led to the Feynman diagrams for particle interactions and to the work that won the Nobel award.

The book swarms with delightful glimpses of the famous: Einstein, Bohr, Wheeler, Wigner, Oppenheimer, Teller, Pauli, Compton, Fermi, Gell-Mann and many others. (It is said that the California Institute of Technology, where Feynman has taught since 1950, hired Murray Gell-Mann so Feynman would have someone to talk to.) And it was, apparently, a casual remark of one of them, John von Neumann, that gave Feynman a sense of political detachment that he claims has kept him happy ever since. You don't have to feel responsible, the great mathematician told him, for the state of the world. Not for its social and political insanities, perhaps, but trying to understand how nature works on her deepest level is a responsibility — perhaps pleasure is a better word for what Feynman feels — that he surely has not taken lightly. □

Martin Gardner, formerly a staff writer for Scientific American, *is a contributing editor of* Discover. *He is author of many books, the most recent of which is* Order and Surprise *(Oxford University Press, 1984).*

Global matters

David R. Rosseinsky

The Cambridge Guide to the Material World.
By Rodney Cotterill.
Cambridge University Press: 1985. Pp.352. £17.50, $34.50.

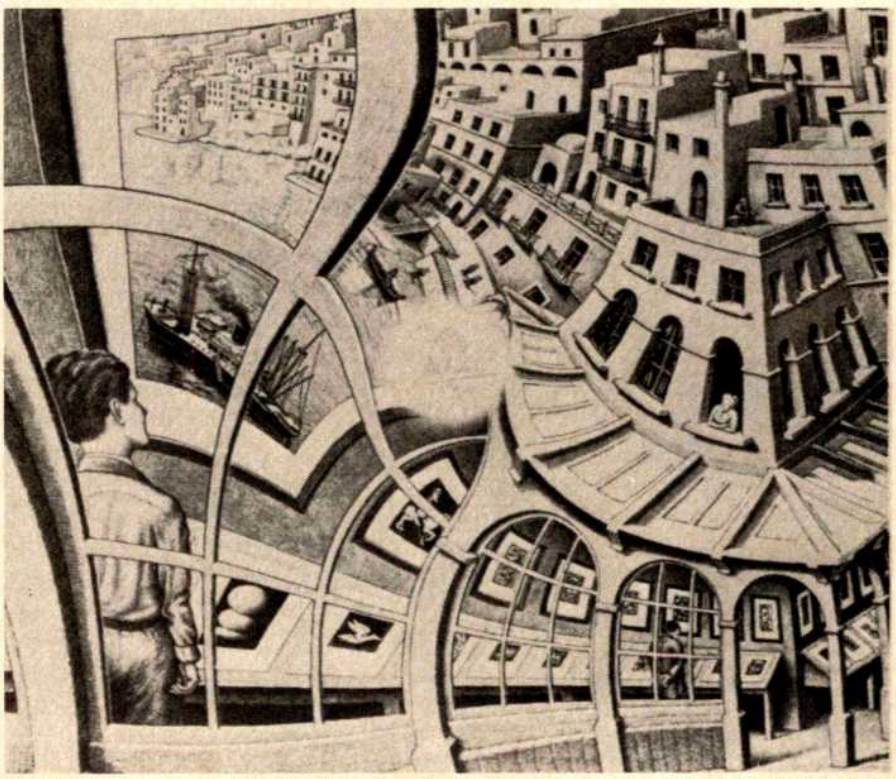
"WE are living in a material world" is the burden of a current popular song, and this inviting tome comprehensively portrays its component materials. While — despite the title — not exactly instruction against excessive monasticism (nor yet a coffee-table book, despite the large and colourful jacket) it possibly does have a message underlying its non-mathematical account of materials. Some are materials as in materials science, that is to say mainly solids (metals, polymers, ceramics and the like, together with the new organic superconductors and kindred exotica) but also included are nucleic acids, cells, living systems and their operation. More than a third of the book is devoted to biological materials, metabolism, nerve and brain operation and so on. Perhaps the philosophical drift of this mechanistic outline lies in the concluding sentence — "The nature of the mind remains a mystery". And thereby may the title be nicely justified.

The level of exposition is a mature one, even though for example the concept of potential energy itself has to be established in terms of uphill and downhill travel. Description of nuclear and atomic structures leads on to the complexity of, for instance, the superconductivity mechanism. Experiment is included insofar as it aids the continuity of description. Lively historical exposition is often followed, and individuals are named at a pleasing narrative pace. And there are many fine illustrations, often in colour.

All is not quite perfect, however: we could do without the entropy death of the Universe (yet again), and on the atomic scale some aspects are just wrong rather than oversimplified. Thus p orbitals are not appropriately represented, the hydrogen bond and electron affinity suffer in verbal compression, the invention of the T-jump rate measurement is wrongly ascribed and so on. It is a measure of the book's overall strength, however, that the description of materials, which is the author's *métier*, survives such shortfalls in the physics and chemistry at the beginning.

What will be the readership of such a book? Certainly established physical scientists will benefit (and perhaps feel smart at spotting the occasional slip). But *all* enquiring amateur or professional scientists and technologists of whatever origin or age should enjoy it, and possibly the eye-catching jacket will fulfil the role of the Venus fly-trap depicted there. The price is such as to encourage private investment, and who knows, even as a coffee-table book it could do good by stealth. In Britain the invaluable campaign for "Women into Science and Engineering" goes under the acronym WISE, and for a WISE education, no Material Girl (of the pop song) should be without a copy. □

David R. Rosseinsky is Reader in Physical Chemistry at the University of Exeter.



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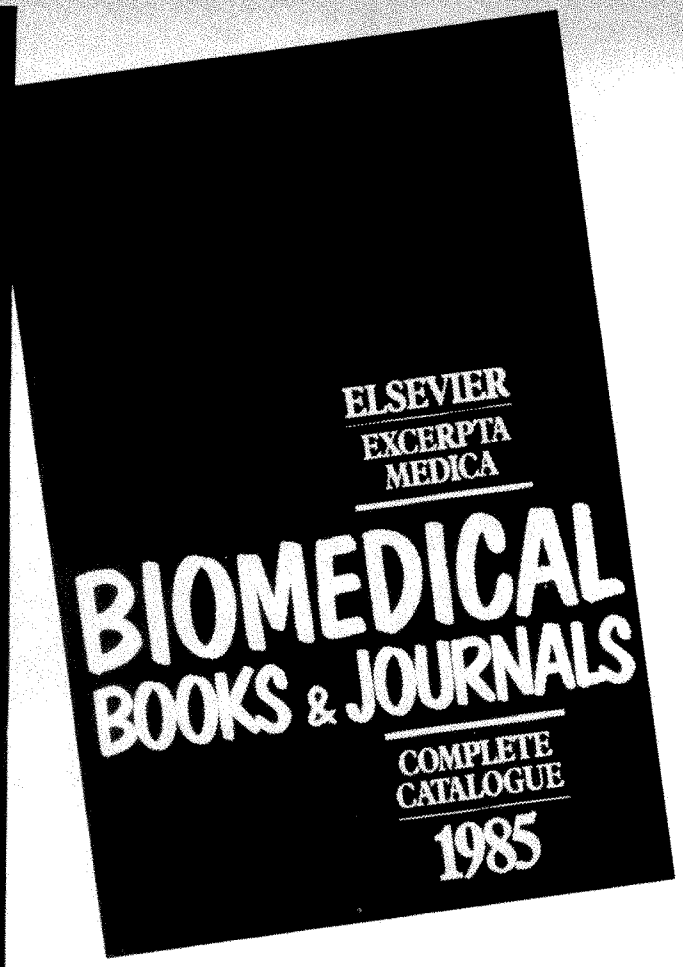
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The foot inaudible and noiseless

David Park

Time's Arrows: Scientific Attitudes Toward Time.
By Richard Morris.
Simon & Schuster: 1985. Pp.240. $17.95.

IN THE philosophies of nature that most scientists seem to work out for themselves, time is something of a dark area. Time has extension analogous to space, but the impossibility of experiencing any instant more than once defies analogies — we face great difficulties if we try to define even so simple a word as "now" without tautology. An odd fact is that all this doesn't seem to matter. To use a concept such as *p*H or electric potential we need to understand more or less exactly what is meant, yet we readily navigate the shoals of time even if we cannot explain what we are doing. Our sense of time passing defies explanation in rational terms (how fast does it pass, then?); but we understand perfectly what we are doing when we use a clock.

This being the case, it has often been felt that there is some need for a further explanation of time, and yet in the absence of any practical difficulties one does not quite see what needs to be explained. In *Time's Arrows* Richard Morris adopts a reasonable programme: "We must discover what science can tell us about time before we can know what the relevant philosophical questions are". His remarks on the ancients are mercifully brief. The eye picks out the phrase "the philosopher Pythagoras" and again "Ancient peoples believed that time was cyclic in character" on an early page, and prepares to close in sleep, but pushing on we arrive at Galileo and the incorporation of a time variable, for the first time, in the description of physical phenomena (in this case free fall). Now we are safely in scientific territory. I do not want to encumber this review by enumerating the author's errors, but will mention three examples of the kind of thing that a reader ought not to have to bother with in reading an otherwise illuminating discussion.

- "Galileo was the first to realize that the acceleration of these [falling] bodies was a function of time." Since this remark is made in the context of motion with constant acceleration, I can only attribute it to some demon in the pen.
- "Furthermore, Aristotle said, the velocity at which an object fell was proportional to its weight." This is indeed the conclusion that was widely drawn from passages in Aristotle (for example *Physica* 215[a] and 216[a]), but these make no mention of falling bodies and "Aristotle said" is hardly the way to summarize them.
- In discussing free fall Morris attributes to Galileo the formula $d = \frac{1}{2}at^2$. Galileo never assigned a numerical value to acceleration and wrote no such formula. He did state that d varies as t^2; this is an immediate consequence of the fourteenth-century mean velocity rule. It would have been an interesting paragraph on the history of science if Morris had discussed the fact, surprising today, that the mediaeval scholars did not seek to apply their mathematical formulae to the natural world.

The next chapter is on "Calculus and the Idea of Determinism". After removing the sentence "Today, all of physics and most of higher mathematics are based on calculus", and placing it like a fishbone on the edge of the plate, the reader finds an excellent account of how the invention of the calculus made it possible to extrapolate present motions into the past and future so that the trajectory of a body, as a whole, could become a subject of study. The implications of Laplacian determinism are carefully discussed, though one would have hoped for at least a brief mention of the qualifications of the idea which the researches of the past decade on highly unstable motions have made necessary.

Morris next devotes two chapters to the enormous expansion of the old time-scale stemming from the work of Hutton and Lyell in geology, and Darwin and Wallace in biological evolution, and then moves on to modern physical science. The arrows of the book's title are five in number: the thermodynamic arrow of increasing entropy, the direction of cosmic expansion, the time-asymmetry of weak interactions, the electromagnetic arrow and the psychological arrow. The electromagnetic arrow, which requires that retarded solutions of the wave equation are the only ones to be retained, is the subject of an especially sensible discussion emphasizing that, in a universe containing absorbing matter, either a diverging or a converging wave can be analysed in terms of either advanced or retarded functions; the predicted phenomena are the same in either case, and so the distinction lies outside physics in the mind of the analyst. The psychological arrow involves a real puzzle: how can one understand from all we know of time why consciousness singles out a single instant, now, for its attention?

The last chapters cover the fairly familiar territory of relativity and cosmology. As a whole, this is a somewhat uneven book, serious and thoughtful in places, jejune in others, disfigured by careless errors, but finally, I think, succeeding in what the author sets out to do: to suggest to the reader some of the thoughts that come to the mind of a scientist invited to focus on the word "time". □

David Park is a Professor in the Department of Physics and Astronomy at Williams College, Williamstown, Massachusetts.

But is it art?

Andrew Dearing

Creative Computer Graphics.
By Annabel Jankel and Rocky Morton.
Cambridge University Press: 1984.
Pp.143. £15.95, $29.95.
Art and the Computer.
By Melvin L. Prueitt.
McGraw-Hill: 1984. Pp.246. Hbk $39.95, £34.75; pbk $29.95, £26.25.

"COMPUTER power" used to refer to the rate at which machines digested numbers. The users were, in the main, scientists, and they generally viewed developments in terms of a new machine's ability to solve bigger problems faster than before. Graphics devices were useful for checking results, but were rarely seen as more than that; there were thought to be better things to do with an expensive computer than use it to draw pictures. Then the price of computers fell, and we finally realized that we could control and understand the computer's calculations better when we could see the problem and the results directly. Equally, quality graphics created a market for personal and small business computers, reaching those for whom numbers held no great appeal, and allowed people working in design, manufacturing and art to discover a new medium with which to express their creativity.

Being creative involves more than producing striking imagery, however. These two books attempt in different ways to examine how computer graphics can be used in this way, and why difficulties are encountered in achieving the desired effects, while presenting a wide selection of pictures that have been produced using the medium.

Jankel and Morton survey many aspects of modern computer graphics technology in order to portray ways in which graphics images can express and communicate ideas, or emulate the natural world. Early chapters trace the history of the discipline, and describe how an object can be modelled within the computer and how these models are converted into images on the screen. Their treatment is non-mathematical, and the novice will have no difficulty following the thread of ideas. Accompanying pictures, which throughout are of the highest quality and generally in colour, complement the text with a refreshing competence and clarity; it would have been all too easy for the authors to rely on the vividness of the pictures to compensate for an anodyne commentary.

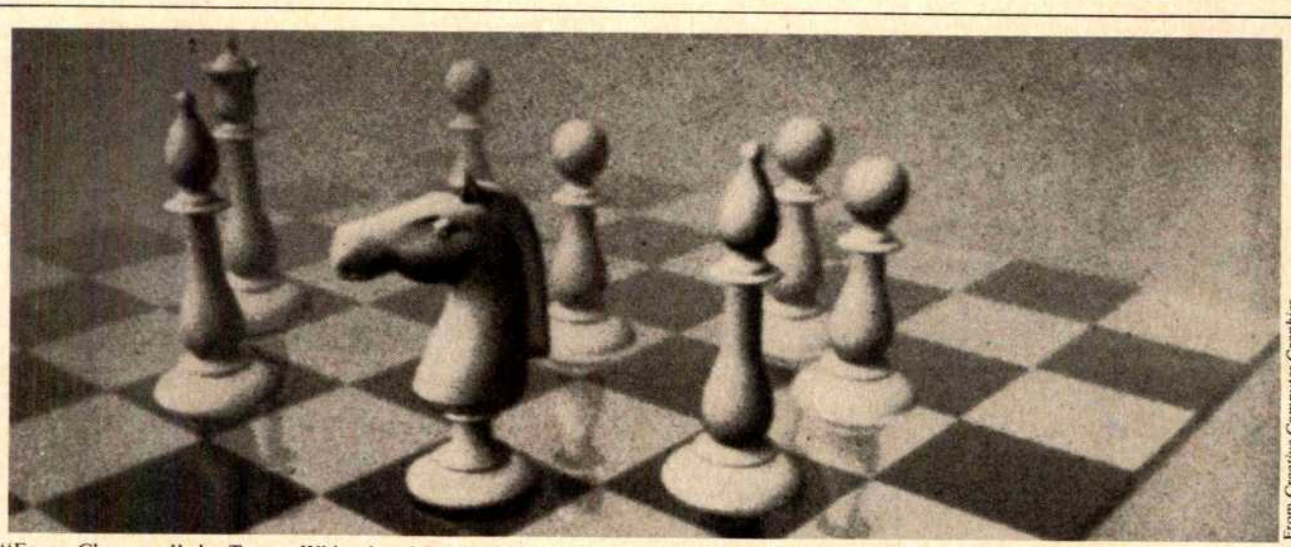
Subsequent chapters are devoted to the ways in which computer-generated images are being applied to science, design, art and the media, and used in home computer programs and video games. The authors pay much attention to the technical and human problems of realizing the technique's potential, and there are some amusing insights along the way. One story that appears to be almost apocryphal describes how an attempt to enter a computer-generated print for a New Jersey competition was rejected in 1969, but accepted the following year when the artist presented the same piece as a hand-made silk screen. We often expect too much too soon, and technical novelty must be studied in terms of what is already known, not presented in isolation, before the artist knows how to use the medium and the viewer can understand the context within which he should see the results. Certainly, Jankel and Morton demonstrate that creativity remains the product and responsibility of the craftsman. In spite of the argument that such creativity resides in the programmed sequence of code that produces the image, the best results seem to be those in which computer graphics is used to complement and enhance material obtained using other techniques.

In *Art and the Computer*, Prueitt concerns himself specifically with computer graphics as an art form, and most of his book is used to present a gallery of pictures representative of recent work. He too describes the methods used to generate the images, and analyses their potential as tools for the artist, but somehow neither his pictures nor the commentary really succeed in showing that computer art is yet on a par with more traditional work. Interesting topics are introduced — for example, Prueitt notes how visual appeal often depends on precise use of colour — but he fails to develop the opportunities he has created, and merely repeats his contention that the results can be considered to be art.

It is, however, not sufficient to define art simply as "creative work produced by a person", since that does no more than make us ask what the author means by "creative", and that question is not answered in the book other than by encouraging us to look at the results for ourselves. Yet, although the work reproduced here is twice claimed to be representative of the finest computer-generated art, by its greatest practitioners, over half of the pictures are by the author himself, and are mostly variations on a theme of broken symmetry, resembling pictures viewed in a kaleidoscope or drawn with a child's epicyclic toy. While they show clear technical skill, there is too little sensitivity to the need to define where the art has contributed something new.

Neither of these books offers much that is new to the specialist. Nonetheless, Jankel and Morton have succeeded in portraying the creative use of computer graphics in a way that will interest both layman and professional. Their book should remain an accurate snapshot of the state of this particular "art" in the mid-1980s. □

Andrew Dearing is currently Head of the Molecular Graphics Group at Shell Research Laboratories, Sittingbourne, Kent.



From *Creative Computer Graphics*

"Foggy Chessmen", by Turner Whitted and David Weimer, produced using scanline algorithms (rather than ray tracing) to produce the shaded effect. Scanline algorithms cannot cope with reflections, and the mirror chessmen are the original ones turned upside down and toned down to give the effect of reflection in a marble chessboard.

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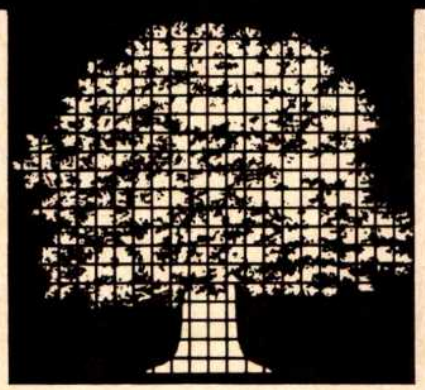
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Imagined worlds: visions of theoretical physics

David Bohm

Imagery in Scientific Thought: Creating 20th Century Physics.
By Arthur I. Miller.
Birkhäuser: 1985. Pp. 320. $24.95.

THE ultimate origin of creative developments in science, art and in other areas of life will probably remain a mystery, no matter how far our knowledge may progress in the future. Nevertheless, it seems reasonable to suppose that a great deal of light can be shed on the subject by a careful analysis of the process in which new ideas develop in actual historical case-studies. In *Imagery in Scientific Thought* Arthur Miller does this for the field of science, tracing the relationship of creative thinking and the construction of new scientific concepts from pre-scientific knowledge. To this end, he focuses on the work of Poincaré, Einstein, Boltzmann, Bohr and Heisenberg, because these physicists were specifically influenced in their research by their consideration of the nature of thinking itself.

In the book, Miller makes the key point that the major change that took place through the work of these scientists was the development of a fresh attitude towards mathematics and the imagination. Thus, Poincaré's view on the subject was that Euclidean geometry arose necessarily from man's earliest thought because it was needed for his survival. For Poincaré this implied the basically conventional nature of the axioms of geometry. To obtain real content, they have to be enriched with lower-level physical hypotheses. The introduction of such hypotheses is through *invention*, and this comes out of an intuition that cannot be defined explicitly but must be felt. This is a kind of Baconian inductivism with gaps supplied by intuitively guided inventions. Physical theories then express in the most convenient manner the "true relations between real objects" along with the invariant laws pertaining in these relationships.

Einstein adopted much of Poincaré's approach, and explicitly expressed his debt to Poincaré in calling attention to the need to "pay strict attention to the relation of reality and our concepts". However, he rejected Poincaré's *a priori* organizing principles along with his closely related assumption of a fundamental role in our thinking for Euclidean concepts. Rather, he regarded concepts in general as admissible "only to the extent that observable facts can be assigned to them without ambiguity". Thus, for Einstein, the question of whether Euclidean geometry is valid was basically *physical* (for example, that of whether it is possible to attach the Euclidean concept of length to a quasi-rigid measuring rod in an unambiguous way).

Einstein considered science to be essentially a refinement of everyday thinking, in which images are of course involved. He felt it necessary to analyse serious thinking (in contrast to "dreaming") as a series of such images ordered by a recurrent image that constantly turns up. Such an ordering element can relate many different series of hitherto unconnected elements, and this ordering element he regarded as a concept.

Though he seems to have started from Poincaré's approach of building a theory step by step by posing hypotheses to explain the experimental data, Einstein realized by the end of 1904 that the "known facts" were too restricted to solve the problem of an electromagnetic world picture. Instead he shifted to an appeal to general axioms and theories of principle, similar in structure to those of thermodynamics. These were the principle of relativity of motion and the principle of the invariance of the speed of light. Here, Einstein's thought was affected by the views of Boltzmann. For Boltzmann, our mental pictures, which he regarded as abstractions from objects actually perceived, must be clearly and unambiguously imaginable. A striking example of this is Einstein's highly imaginative picture of an observer who moves along with a light wave and sees only a static configuration of fields. The implication of this picture violated Einstein's physical intuition that the electromagnetic field is inherently dynamic, and eventually led to his taking the invariance of the speed of light as a basic principle.

Through the consideration of the properties of dynamos (as well as in other ways) Einstein was then led to regard Lorentz's idea of a local time in terms of which Maxwell's equations were invariant (to first order in v/c) as similarly indicating a general principle, which was the relativity of simultaneity (and which held to all orders in v/c). Here, Einstein was clearly guided by the notion that our ordinary concepts of time can no longer be related unambiguously to the experimental facts when we consider the full implications of electrodynamics (as he showed with the aid of various hypothetical experiments). So he was ready to go against ordinary intuitive experience when he felt this to be necessary, but he still insisted that concepts have to be *some* kind of abstractions from our pre-scientific perceptual experience which must be unambiguous in the senses indicated above.

This brings us to the contributions of Bohr and Heisenberg. Their analyses of the meaning of the quantum theory indicated to them that there are limitations on the possibility of unambiguous intuitive visualizability of the properties of matter at the quantum level. Bohr emphasized the need to separate the space-time and the causal descriptions. This implied that the ordinary imaginative pictures can be retained, but that they have to be *ambiguously* related to experimental facts. Here, perhaps, is the fundamental difference between Bohr and Einstein, who, as we recall, insisted on the need for the possibility of an *unambiguous* relationship between concepts and the experimental facts. In my opinion, it is unfortunate that Miller does not bring this point out in the book, as it is of crucial importance in understanding the split that eventually came about between Bohr and Einstein, a split that profoundly affected the development of quantum theory and relativity.

Heisenberg's initial approach was, in contrast to that of Bohr, to insist that our customary intuitive visualization (*Anschauung*) could not be extended into the atomic domain at all. Miller follows the further development of Heisenberg's approach in some detail, to show how it led eventually to a new notion of intuition and imagination, which Heisenberg called *Anschaulichkeit*. This referred not to ordinary visualization, but rather to the imaginative display of the meaning of the mathematical formalism (which latter was by then regarded as the most direct and fundamental way possible of putting the truth about nature). This mode of visualization is now most commonly encountered in the Feynman diagrams which do not correspond to reflections of actual physical processes, but which serve primarily to enable physicists to think imaginatively about the meaning of the mathematics. As Heisenberg himself said, this is essentially an extension of the Platonic (and perhaps Pythagorean) approach. It may be summed up in the dictum of Jeans: "God is a mathematician".

If one looks at how modern theoretical physics is actually done, one can see that Heisenberg's approach now dominates, not only in particle physics but also in cosmology. Miller's book provides important insight into how the whole way of doing theoretical physics thus altered radically in a comparatively short time. This insight could, however, perhaps have been extended if the author had brought out, even if only briefly, how several centuries ago a similar change occurred, culminating in the Newtonian approach. To have done this would have indicated that what constitutes commonly accepted scientific practice has constantly been changing, and that it may therefore change again in an equally fundamental way. Tacitly to regard the currently accepted way of doing physics as the last word on the subject is to encourage the same sort of absolutist conclusions that took hold of the Newtonian way of doing physics, and thus help to ossify the current approach too.

The latter part of the book is a survey of various attempts of modern psychologists to account for the creative development of modern physics. It begins with a description of Wertheimer's application of Gestalt

psychology to Einstein's process of thinking in the work that led to the theory of relativity (Wertheimer's work drew upon actual discussions with Einstein himself). Miller then goes on to a broader discussion in terms of new researches based on extensions of Piaget's work on genetic epistemology. Two different versions of genetic epistemology are considered here, the first due to Pylyshin and the second to Kosslyn. Pylyshin and Kosslyn are in agreement in that they develop detailed accounts of how imaginative thought actually proceeds and try to support these accounts on the basis of actual case-studies. Their principal disagreement is on the dynamic role of images. Roughly speaking, Kosslyn assigns a positive predictive power to images, while Pylyshin regards them, in effect, as little more than passive displays of abstract propositional thought.

Although Miller gives what seems to be a fair treatment of this whole subject, I feel that he fails to be adequately critical in that he accepts an assumption that seems to be taken for granted in all the work on genetic epistemology. This is that creativity is primarily to be understood through the development of knowledge, enriched perhaps from time to time by *invention* whose source is (along the lines indicated by Poincaré) ultimately in a mysterious intuition. I would like to suggest that while all of these factors are involved in creativity, its primary source is in *perception*, not only through the senses, but, even more, through the mind (as indicated by the Greek *noesis* and the German *Vernunft*, which latter means intuitive or perceptive reason). Kostler gives an example of this, in the sudden realization of Archimedes that the volume of water displaced by a body is the same as the volume of the body independently of the complex details of its shape. Perhaps we could say that Einstein must have similarly realized somehow that the problem with electrodynamics was essentially the same as with thermodynamics; that is, to find principles that are independent of a detailed dynamical description of all the complex processes that are actually at work in concrete experimental situations. It seems to me that a study of the history of the subject from such a perspective is necessary, if we are to have a proper account of how the mind gets out of the "rut" of thinking in terms of old concepts that have long been taken for granted.

I feel that in spite of certain inadequacies Professor Miller has made a valuable contribution to our understanding of creativity in twentieth-century physics. His book is well worth reading by all those who are interested in the history and philosophy of science, or in the understanding of the nature of creativity and its relationship to the process of thought. □

David Bohm is Emeritus Professor of Theoretical Physics at Birkbeck College, University of London.

From Cambridge to Moscow

Nevill Mott

Kapitza, Rutherford, and the Kremlin.
By Lawrence Badash.
Yale University Press: 1985. Pp.129. $20, £20.

LAWRENCE Badash has dedicated this book to the memories of Ernest Rutherford (1871–1937) and Peter Kapitza (1894–1984). The story of the relationship between the two men is well-known. Kapitza, a brilliant young pupil of A.F. Joffe in Leningrad, lost his wife and two children in the influenza epidemic of 1921, and, in an effort to rescue him from his deep grief, friends arranged a trip abroad. They were successful; Kapitza knew where he wanted to go and arrived that year in Rutherford's Cavendish Laboratory. The Mond Laboratory was built for him and a Royal Society chair became his. There he developed equipment for producing strong magnetic fields and low temperatures, work on which was to culminate in the award of a Nobel Prize in 1978. The friendship between the two men must have been very deep, particularly as these branches of physics were far away from Rutherford's beloved nucleus.

Kapitza, as I remember, used to boast that he was the only Soviet citizen whose passport was endorsed for unlimited travel in and out of the Soviet Union. In 1934 he went with his second wife Anna to the congress in Leningrad which celebrated the one-hundreth anniversary of the birth of Mendeleef. They took their car to Bergen, and motored through Norway and Finland. I remember this, as I was invited to the conference, probably because I had helped Frenkel with the English version of his book on liquids, and Kapitza suggested that my wife and I should join them in their car (in the event there seemed not to be enough room for us and the luggage, and we went by boat). Once there, Kapitza was told that his country needed him and he must stay. Anna went back to Cambridge, joining him with the children more than a year later.

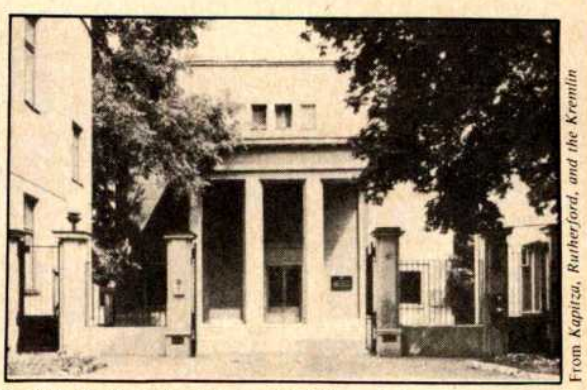
In the book Badash tells the story of Rutherford's efforts to help him, reproduces many of Kapitza's agonized letters to his wife and to Rutherford, and records how eventually much of the Cambridge equipment was bought by the Soviet government, and how, two years later, he was able to settle down to work in his new Institute for Physical Problems of the Academy of Science. He had the help of two of his technicians from Cambridge, who spent several years with him in Moscow.

Kapitza's misery in his first year, which might have seriously endangered his health, was not because he was unwilling to work for his country; he always protested that he wanted to. It was because, until the Institute was ready, there was nothing he could do. To be cut off from work was dreadful for him. If he could have returned to Cambridge, supervised the transfer of equipment to Moscow, finished off certain researches and indeed been treated like a loyal Soviet citizen, he would not have complained, or at any rate not so bitterly. But — and this is what made life intolerable — he was given no trust. People were afraid to talk to him and so he had few friends. An official was detailed to look after him, and promised much, but when Kapitza's winter clothes were posted to Moscow from Cambridge he could not even get the parcel through customs without Kapitza having to pay a huge import duty. Kapitza was denied Western newspapers, but letters to and from his wife seemed to get through, as did telephone calls. I was once in R.H. Fowler's house when a call came through from Kapitza, which lasted a very long time. Apparently he could at least do that, and trunk calls must have been cheap.

A letter from Kapitza to Rutherford in the early days of the Institute records vividly the difficulties of doing research at that time under the Soviet system:

> You see Soviet industry is growing at a terrific rate and all is done to make its growing organised and well planned, so that all the system of supply and production is also well planned and organised. But the supply of the factory, which is operating according to a definite plan, must be anticipated in details at the beginning of the year, and evidently represents its demands in very large figures. Such a system is indeed quite unsuitable for the supply of laboratories. I have written and spoken to the





From *Kapitza, Rutherford, and the Kremlin*

Peter Kapitza (about 1925) and the Institute for Physical Problems, Moscow.

authorities that the labs must be supplied in a different way, and I think that the people here begin to recognise that the system of supply must be altered for the scientific institutions. But, at present, if for instance we require four bars of phosphor bronze, the need of which we had not anticipated in the beginning of the year, we must get it as an exception and the permission of the sub-secretary of the Heavy Industries is required and this means a lot of correspondence, which is equally extensive if we require 10 kilograms, 10 tons or 10 loads of stuff. And so even if the Institute is given sufficient money, and even if the industries are quite decent, we are actually very badly supplied.

He believed, however, that things would get better. But later he wrote:

My colleagues the scientists are very scared of me and behave like pigs. My institute is attached to the Academy of Science, of which I am not a member but they govern my institute. The president is Karpinsky, he is ninety years old. During meetings of the Council he sleeps with a happy and kind smile on his face.

It is for insights such as these, showing the reactions to Soviet bureaucracy of a great man who had experienced Cambridge, that I recommend this book. Parts of it are compulsive reading. How much the bureaucracy has changed since then is not discussed but in any case Kapitza came to terms with it, became a Hero of Socialist Labour, received several Orders of Lenin and was throughout a sane and courageous voice in Soviet science. One wonders if he would have survived the early days without Rutherford's moral support, and Rutherford's efforts — though unsuccessful — to secure his release, which are here described by Badash. Kapitza repaid some of his debt in his "Recollections of Lord Rutherford", published in the *Proceedings of the Royal Society* in 1966.

Kapitza, then, was back to work by 1936. The author remarks that the timing was fortunate, for Rutherford, upon whom he depended so much for aid and encouragement, died in October 1937. □

Sir Nevill Mott is Emeritus Professor of Physics at the University of Cambridge.

Between machination and merit

Robert Fox

The Beginnings of the Nobel Institution: The Science Prizes, 1901–1915.
By Elisabeth Crawford.
Cambridge University Press: 1985.
Pp.281. £22.50, $34.50.

A FEW days after Alfred Nobel's death in December 1896, his close relatives gathered expectantly for the reading of his will. They were in for a shock. For Nobel had bequeathed to his family a mere one million crowns. The rest of his vast estate, amounting to 30 times that amount (over £1½ million), was to be invested to provide five annual prizes, for physics, chemistry, medicine or physiology, literature, and what Nobel (a convinced pacifist, despite the fortune he had made from explosives) called "contributions to the fraternity between nations". Predictably, the will was contested. But the adjustment that was made in favour of the family was meagre, and in 1901 the first prizes were awarded essentially in accordance with Nobel's wishes.

Elisabeth Crawford traces the difficult birth of the Nobel system and the labyrinthine processes by which the awards were made in physics and chemistry between 1901 and 1915. The sheer size of the prizes, each of them worth about 30 times the annual salary of a university professor, was enough to ensure intense public interest. And the element of spectacle was further enhanced by national as well as personal rivalries which made it difficult for the brash newcomer to integrate with the rather chaste reward system of European science as it existed at the beginning of the century. Tradition demanded, of course, that the cruder rivalries should be concealed. But Crawford penetrates this facade to excellent effect. Drawing on her intimate knowledge of the relevant Nobel archives (open since 1974, in accordance with a new 50-year rule) and of the context and content of Swedish science in the early twentieth century, she casts radically new light on the complex interaction between the dispersed international networks of nominators and the subject committees in Sweden.

Few readers will be surprised by Crawford's demonstration of the keen opposition between the various national traditions in science, for, by 1900, science was emerging as a battle-field on which the European powers were only too ready to try their strength, not least with the disturbing new force in research, the United States. The French, it seems, were especially prone to nominate their fellow-countrymen, far more so than the Germans, who have a commendably low score on Crawford's ingenious index of chauvinism. (Britain's record was mixed, with the physicists appearing distinctly more chauvinistic than the chemists.) However, Crawford's evidence suggests that it was the Swedes themselves who injected the greatest element of competitiveness. Here, the main antagonists were Svante Arrhenius, already distinguished internationally for his work on the theory of solutions, and the mathematician Gösta Mittag-Leffler. The two men were poles apart in their political sympathies and in temperament and lifestyle. Arrhenius was liberal, popular and modest to the point of insecurity about his scientific abilities, whereas Mittag-Leffler was conservative, flamboyant and more respected than liked. As Crawford shows, Arrhenius and Mittag-Leffler intervened rather irregularly, but whenever they felt moved to support a personal preference, or to thwart the candidate of a rival, they acted with rare zest.

In this running confrontation, Arrhenius had a notable early success when he saw to it that the first prize in chemistry went to J.H. van't Hoff, whose work on electrolytic phenomena complemented his own. In 1909, Arrhenius successfully backed another ionist, Wilhelm Ostwald, and, using more negative tactics, he managed to delay an award for his rival Walther Nernst until 1921 (15 years after the first nominations for Nernst had been received). Mittag-Leffler, by contrast, saw his main mission as the advancement of mathematical physics. It was he who secured at least a share in the physics prize for H.A. Lorentz in 1902. And it was only misfortune which denied him his greatest triumph: his long campaign in favour of Poincaré had still not succeeded when Poincaré died in 1912.



Ann Ronan

Arrhenius — "modest to the point of insecurity about his scientific abilities . . .".

These episodes and the intolerance they imply are far from edifying. But generally Crawford's book is reassuring. It provides ample evidence that although animosity, national interest and the stage-managing of nominations all played their part, scientific merit was nevertheless consistently rewarded. It is arguable that a blunder was made in 1908, when the French experimentalist Gabriel Lippmann emerged as a successful compromise candidate, following Mittag-Leffler's mishandling of his own campaign in favour of a joint award to Planck and Wien. But while Lippmann was no Planck, even this decision rewarded excellence of a kind. Like the overwhelming majority of the decisions discussed by Crawford, it seems a beacon of wisdom when compared, say, with the award of the first prize in literature to the poet Sully Prudhomme as a sop to French pride. All told, the physicists and chemists of Europe and North America come out of this intriguing and well-told story with a good deal of credit. □

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On trans-scientific turf

Owen Gingerich

Beyond Velikovsky: The History of a Public Controversy.
By Henry H. Bauer.
University of Illinois Press/Harper & Row: 1985. Pp.354. $21.95, £24.25.

"THE thousands of words spilled to refute and condemn his theories gave them a sort of status, even if only that of divinely inspired nonsense." So the London *Times* summed up the controversy surrounding the work of Dr Immanuel Velikovsky when he died in November 1979.

Basically, Velikovsky claimed that in the ages before Christ a large body had been ejected from Jupiter and had gone into orbit around the Sun, eventually to become the planet Venus. On its way, it came close enough to the Earth around 1500 BC to cause the pharonic plagues of *Exodus* and may other miraculous phenomena recorded in the Old Testament, including the day the Sun stood still at Gibeon. Relying on eclectic interpretations of world-wide mythology, Velikovsky amazed his readers with his erudition and dismayed astronomers by his flagrant disregard for Newtonian mechanics.

The hue and cry against Velikovsky's theory came primarily in two waves. When his principal work, *Worlds in Collision*, appeared in 1950, heralded by sensational abridgements in *Harper's, Colliers* and the *Reader's Digest*, astronomers stirred up such a tempest that Velikovsky's place in the best-seller list was assured. Having learned that any publicity is good publicity, the scientists quickly lapsed into indignant silence, hoping that the interest in Velikovsky's theories would atrophy under their careful lack of attention. Their scheme may well have worked except that Velikovsky was "rediscovered" by the anti-establishment generation of the 1960s, for whom he became something of a folk hero. The scornful attacks of the early 1950s, and especially tales of a threatened textbook boycott against his publisher, Macmillan, helped build up an image of a scientific martyr, a modern-day Galileo alternately jeered and ignored by the entrenched scientific establishment.

Thus, by 1974, several of us agreed that a forum should be provided for Velikovsky to meet his scientific critics, and for the scientists to put on the record a clear statement of their objections. The actual encounter, at a meeting of the American Association for the Advancement of Science in San Francisco, proved to be a memorable non-meeting of minds, with a highly polarized audience full of partisans unlikely to be swayed by arguments or counter-arguments from either opposing camp. Scientists who had advocated silence on the matter went away with an "I told you so" attitude. Nevertheless, this second wave of criticism was harder for Velikovsky's supporters to cope with, and Velikovsky felt particularly stung by the witty and articulate attack made by Carl Sagan. In colourful terms, Sagan described such things as the "fly ablation problem", that is, the difficulty of flies originating on the Venus–comet in entering our atmosphere just in time to cause the pharonic plague of pests.

Today, although Velikovsky's catastrophism is rapidly being forgotten, the fierce controversy that swirled for three decades can still teach us something about the nature of science and trans-scientific challenges. It is such questions that Henry H. Bauer, Dean of the College of Arts and Sciences at the Virginia Polytechnic Institute, has explored. A personal and somewhat idiosyncratic account, *Beyond Velikovsky* contains material to infuriate or at least exasperate everybody. Nevertheless, it is the most balanced and informative analysis yet to emerge from the whole affair.

Bauer argues that science does not find absolute truth, but only a high degree of probability. This prevents scientists from claiming that any particular sequence (such as Velikovsky's) is impossible, only that it is staggeringly unlikely. However, Velikovsky never claimed that his scenario was probable, only that it happened. Hence, counter-arguments that it was improbable completely missed the point. Velikovsky was staking his claims on an entirely different turf — "trans-scientific" in Bauer's terminology — and so the normal type of scientific argument lost its force against Velikovsky and his supporters. In their frustration, scientists were frequently reduced to appeals to authority (their own, of course), which went directly counter to their own claims of how science worked.

Unreal Science

SIR,—As a qualified and practising scientist I wish to dissociate myself from your editorial of April 12. You allude to Geller and Velikovsky with terms like "nonsenses", "beliefs beyond science", and "pseudo-scientific ideas", and contrast these with "real science" and "science based on conventional ideas about the way a scientific investigation should proceed", and you declare that "scientists want to fight this distressing drift from the scientific way of thinking".

History is littered with ideas shown to be false by people bold enough to question their contemporary conventional science, often in the face of personal ridicule and even persecution.

Presumably you class Einstein's

Passions run high — a letter in Nature, *28 June 1974, in response to an editorial on Velikovsky.*

Velikovsky, too, was frustrated by the resistance from the scientific community, so he consulted his neighbour at Princeton, Albert Einstein, about the status of his theory. Einstein assured him that it would be better if the theory could *predict* something. Thereafter Velikovsky searched his writings and came up with a series of "advance claims" that he had made, ranging from high temperatures on Venus to radio noise from Jupiter. Unfortunately, Einstein had not made it clear to him (and thus to Velikovsky's followers) that predictions have to be made for the right sorts of reasons to be persuasive. The principles must fit with the coherence of scientific understanding, and they should not be single *ad hoc* instances incapable of supporting further, related predictions.

Bauer analyses both Velikovsky's physical reasoning and his advance claims, and both are found wanting. Although Velikovsky's scenario cannot be denied outright, it is relatively easy to show that his understanding of physical principles was hopelessly faulty. "There is rarely a certain way of identifying a crank", Bauer cautiously asserts, and only after 150 pages does he label Velikovsky one in the sense of a person "enthusiastically possessed by a particular crotchet or hobby". But in a devastating chapter entitled "A Nontechnical Case against Velikovsky" he leaves no doubt that the enthusiastic Dr Velikovsky was also a crank in the more pejorative sense of the word.

Nor do Velikovsky's opponents escape Bauer's criticism. "In retrospect", he writes, "it seems clear that Velikovsky's critics did not grasp the magnitude — or perhaps even the nature — of the task of discrediting Velikovsky in the eyes of the public at large". Bauer charges them with errors of commission — arguing *ex cathedra*, with ridicule, *ad hominem* — as well as with errors of omission. In the latter category is, primarily, the failure to capitalize on the unoriginal character of Velikovsky's scheme.

There is, however, one logical path that, curiously enough, Bauer has not explored. Although science cannot prove that a Velikovskian scenario is impossible, it might well prove that it did not happen. At the San Francisco confrontation, Peter Huber provided evidence from Babylonian records that Venus in 1600 BC behaved just as it did in the centuries immediately before our own era. I remarked at the time that if Huber was right (as I certainly believe), Velikovsky's scenario was finished. Velikovsky tossed one of his *ad hominem* barbs at Huber, labelling him a self-confessed amateur in Babylonian. Huber, who is one of the world's leading statisticians and a redoubtable cuneiformist on the side, quickly proved on the stage how ignorant Velikovsky himself was of cuneiform languages. The Velikovskyites were naturally reluctant to admit defeat by a single stroke, so they have since worked hard to discredit the Babylonian data,

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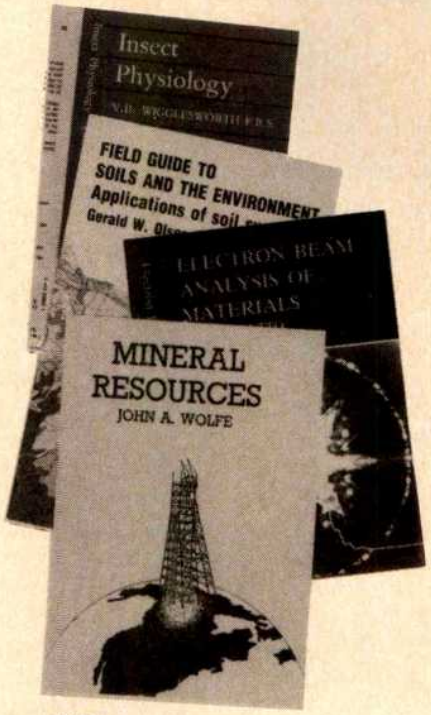
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which were unavoidably contaminated with copyists' errors (and hence Huber's statistical approach). Ambiguous data often characterize the cutting edge of science, and discussion of the point would have added another dimension to Bauer's analysis.

With the decline of interest in Velikovsky, another, surprisingly similar, challenge is facing the scientific establishment: so-called "Creation Science" whose extreme form calls for a 6,000-year-old Earth geologically shaped by Noah's flood. Fossil footprints of a giant humanoid beside indisputably genuine dinosaur prints in the rocks of the Paluxy Creek, Texas, are taken quite seriously by many of these fundamentalist, Biblically-literalistic people. Bauer's closing chapters on "Means of Persuasion" and "Some Realities about Science" offer sound advice about pitfalls to be avoided in these "trans-scientific" debates, where probabilities, values and desirability are at stake. How successfully his analysis can be brought to bear upon this new challenge remains to be seen. But arguing that the creationist ideas have been heard before does not seem to be a cogent strategy. Rather, we must argue that even if science is "never completely final, always subject to question and doubt" (as Einstein put it), nevertheless it is of a piece, not easily shredded, and the process of building this coherent tapestry is what science is all about.

Beyond Velikovsky is awkwardly repetitive, not as complete as it might appear at first glance and in its own way rather too polemical. Its system of references, numbered but unattributed in the text itself, is seriously distracting. Nevertheless, the account is detailed, thought provoking, and the best yet written on a bizarre episode that continues to smoulder in the annals of science. □

Owen Gingerich is Professor of Astronomy and the History of Science at the Harvard-Smithsonian Center for Astrophysics, Cambridge, Massachusetts.

Continental epic

Robert M. Hazen

Landprints: On the Magnificent American Landscape.
By Walter Sullivan.
Times Books, New York: 1984. Pp.384. $22.50.

"THERE is no epic tale to compare with the account of how the North American continent came to be." So begins *Landprints*, one of the finest popular earth science books to appear in decades. In this recounting of the 3,500-million-year evolution of the mountains and valleys, rivers and deserts of North America, Walter Sullivan, the science editor of the *New York Times*, has achieved a masterly blend of geomorphology, historical geology, geophysics, structural geology, palaeontology and geodynamics.

The book consists of 37 short, self-contained essays on the principal features of the American landscape. Inspiration for this geological feast came from the author's frequent transcontinental flights; as Sullivan observes, many of the continent's landforms are best viewed from the air. The first two dozen chapters, therefore, focus on striking geomorphological features — parallel ridge systems, meandering rivers, volcanic craters and cones. The White Mountains, Wyoming basins, Hawaiian Islands and the Grenville Province are among the many areas which are examined first in terms of their distinctive aerial appearance, but then also from the standpoint of their underlying geological structure and history. In this way Sullivan gradually introduces current views on plate tectonics, continental subduction, and the regional folding and faulting that are associated with mountain building.

The last third of the book emphasizes the dramatic alterations of the Earth's surface imposed by man. Farms and mines, cities and suburbia have all left their distinctive imprint on North America, and Sullivan discusses both their geological and environmental significance. He explains why East Coast cities grew up along a former continental shelf margin (the "fall line"); he shows how rock erosion influences soil chemistry, which in turn controls the predominant crops of American farms. From aerial observations of the distinctive patterns caused by centre-pivot irrigation, hydroelectric dams and strip mining, Sullivan proceeds finally to consider the possible consequences of man's actions on his environment.

Landprints is graced by a magnificent collection of photographs and other illustrations. Aerial and Landsat photos — more than 150 of them — display the variety and splendour of North American topography, both natural and man-made. Views of ancient craters, barrier islands, dune fields and the crazy-quilt of America's farms, photographs of road-cuts, regional maps and schematic diagrams of geological processes, all enrich the presentation.

This is a beautifully produced book, too, with clear typography, a felicitous layout and sturdy binding. The writing is stylish and informative, in the best tradition of popular scientific literature; Sullivan brings to his subject an infectious sense of wonder and enthusiasm — he entertains as he teaches. Anyone interested in science, particularly those who travel frequently by air, will be well rewarded in reading this continental epic. □

Robert M. Hazen is at the Carnegie Institution of Washington's Geophysical Laboratory, Washington, DC.

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Earth science in a fabulous place

Peter J. Smith

Atlantis in the Light of Modern Research.
By Zdeněk Kukal.
Elsevier: 1984. Pp.224. $57.75, Dfl. 150.

How the childhood memories come flooding back! The idea of Atlantis was introduced early into my genteel upbringing, presumably as a spur to the imagination much as a spot of breakfast is supposed to set the digestive processes rolling. Plato's lost world beat its competitor, Little Noddy, hands down, losing its status as prime imaginative wonder of the world only under the impact of the higher Blytonian *Island of Adventure* and, later, the more robust yarns of Percy F. Westerman and Arthur Conan Doyle. Besides, in the black-and-white world of the teens, fiction was fiction and fact was fact, but fiction masquerading as fact (as, by then, we all knew Atlantis to be) was strictly for the gullible. When we became adults we put away childish things, did we not?

But no, actually, some of us didn't. N.F. Zhirov of the Soviet Union, reputedly the world's most devoted "atlantologist", is even now engaged in a review of all of the estimated 3,600 works on Atlantis published to date. A Dr E. Sykes is said to edit *Atlantean Research*, a periodical containing such gems as "Atlantis, land of giants" and "Atlantian script". Pierre Benoit wrote the most famous of all novels about the place. And Zdeněk Kukal, a member of the Geological Survey of Czechoslovakia and author of more than 200 legitimate papers and books on matters geological, now presents the results of 20 years' study of the scientific aspects of Atlantis.

What these four representatives of a much larger motley demonstrate, of course, is that Atlantis has a number of distinct faces. There is Atlantis as jumping-off point for harmless fantasy, which sees Plato as an early science-fiction writer. To the extent that fantasy is therapeutic, here surely lies Atlantis's most abiding contribution to the welfare of the human race, although Kukal ignores all that because it is not science. Then there is Atlantis as advanced civilization. But is it likely that there was really, as Plato claimed, a ten-million-strong community that could write, work metals and breed domestic animals many millennia before the time for which there is evidence of such things elsewhere? Kukal thinks not.

That leaves Atlantis as a physical, geographical and geological entity, albeit one that, according to Plato, suffered "violent earthquakes and floods, and in a single day and night of misfortune . . . disappeared in the depth of the sea" about 11,500 years ago. Not that Plato's sea should be taken too literally, it seems, for of the 40-odd possible locations of Atlantis given in the literature (ranging from the United States to the Black Sea and from Iceland to Zimbabwe) almost half are on land. As far as the ocean floor is concerned, however, Kukal has to admit that echo sounding, deep-sea drilling, dredge and grab sampling, underwater photography and bathymetric descents have revealed not one whiff of Plato's place.

From this discouraging beginning Kukal goes on to assess in some detail whether we could just have missed the sunken state (possibly), whether city walls and metal artefacts could have survived 11,500 years of submergence anyway (highly unlikely), whether Atlantis could have been any of the fictitious islands shown on early maps (no), whether it could have been any of today's real islands (geologically impossible) and whether there are any areas of subsidence in the Atlantic or Mediterranean which might qualify (not really). He then considers whether the supposed Atlantis could have been destroyed by earthquake or tsunami (theoretically possible), storm flood (probably not), volcanic eruption (yes), slow tectonic movement (no), cosmic impact (no evidence) or rising sea level (yes, but not "in a single day and night").

There is something faintly ludicrous about all this; it is a bit like mounting a scientific search for the invisible man simply because H.G. Wells happened to write about one. On the other hand, in describing the search for Atlantis, Kukal does manage to impart a lot of earth science — the geology of islands and oceans, the nature of glaciation, the role of sea-level changes, the processes of weathering and much else besides. The means may not justify the ends, but they make a fascinating story all the same.

I began the book with an inclination to mock, but ended up with a grudging respect for Kukal. He has produced a fine and objective refutation of a "theory" that may be inherently ridiculous but is nevertheless important because it has duped thousands of people over the centuries. Atlantis was a deliberate fiction and a particularly successful one at that, for it still attracts attention after 2,500 years. The advanced civilization of Atlantis was Plato's Utopia; its physical and political structure was the best Plato could envisage; and its destruction was by processes known to him. There can be no other conclusion, although one almost wishes that after 20 years of work Kukal could have proved otherwise. Of course, the only people to accept his verdict will be those who believed it in the first place. Those who don't never will. All will continue much as if Kukal had never been. □

Peter J. Smith is Reader in Earth Sciences at the Open University.

Swimming through the cell

Tim Hunt

A Guided Tour of the Living Cell, Vols 1 and 2.
By Christian de Duve.
W.H. Freeman:1985. Pp.423. $55.95, £39.90 per set.

Here is the latest biological work from W.H. Freeman in the Scientific American Library. It spans two volumes, but is a seamless whole and the two jackets are identical except for the words "One" and "Two". Like its predecessors in the series, *A Guided Tour of the Living Cell* is beautifully produced and written, and its author uniquely qualified to show us around. Christian de Duve is one of the great pioneers of cell biology; together with Albert Claude and George Palade, he was justly awarded the Nobel Prize in medicine in 1974 for his "discoveries concerning the structural and functional organization of the cell".

You may remember a film called *The Incredible Voyage* in which heroic brain surgeons operated on the patient by shrinking themselves down to a microscopic size and making their way to the site of the damage in a tiny submarine which was injected into a vein. The operation successfully completed, they escaped by means of the tear duct after many a frightening adventure, the worst an ugly encounter with a macrophage. In his books de Duve shrinks the reader even further, to the point where the very organelles of the cell are as huge caverns to the observer. What is more, we are protected only by a diving suit (a magic suit, it's true, made of the material that coats the leprosy bacillus) as we swim around the cell with the Master as our guide. The idea sounds perilously twee, and when the introduction reveals that these are the books of a series of childrens' Christmas lectures, the heart sinks even further. However, my initial suspicion wore off within a few pages and the idea works brilliantly well — they must have been fabulous lectures!

Not surprisingly, de Duve is at his best describing the workings of the membrane systems of the cell, and particularly in describing lysosomes. As he says "understanding, not description, is the true purpose of scientific exploration", and in this chapter understanding comes as if by enchantment. We are lucky in other ways besides. de Duve's language is plain, his images concrete and familiar, and the text is strewn with wise words. Several times I found myself warming, seeing things said that do not often appear in books of any kind, let alone relatively elementary texts. Other agreeable features of the general style are de Duve's readiness to admit ignorance and to point out

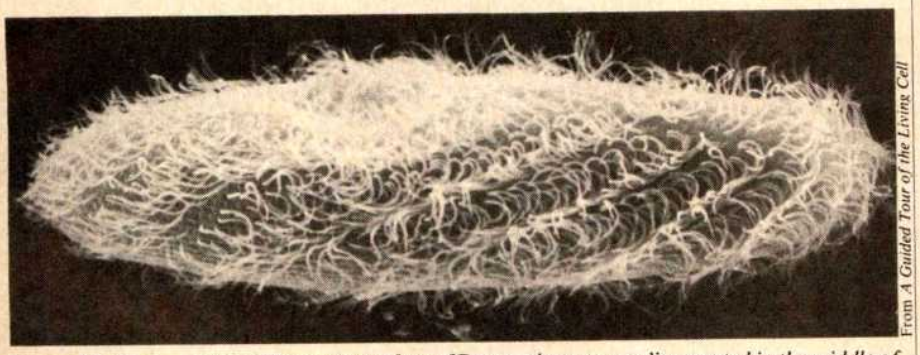
puzzles, and his unfailing grace in explaining the (usually classical) origins of terms.

However, the *Incredible Voyage* approach has its limitations, most evident in the biochemical parts of the books. Very few people really enjoy biochemistry, probably because of its rather abstract nature. It's a subject that's fun to *do*, but not so much fun to read about. de Duve adopts two "outlandish terms" (sic) the "Janus Intermediate" and the "Oxphos Unit". The Janus Intermediate "arises from a nucleophilic attack by an oxygen containing building block (X–OH or X–O$^-$) on ATP or some related energy-rich molecule, which we will designate provisionally as A–B, in order to avoid going into complex chemistry"; later we learn that "Janus...is Mercury and Aquarius all in one". You see? The excellent colour graphics make it more palatable, but whereas other parts of the book repeatedly opened my eyes, I found the biochemistry and bioenergetics not only unilluminating but even baffling in places. I never really did get the Oxphos unit.

In his introduction, de Duve is apologetic about his approach to biochemistry, as if he knew he was taking a bit of a risk and hadn't quite got it the way he would have liked. It would be interesting to know how a non-biochemical reader would fare. It *is* very difficult to know how to deal with biochemistry in a modern account of the cell. Whether you are a strict cell biologist or a full-blooded clone-and-sequence molecular man, enzymes and the reactions they catalyse keep on creeping into consciousness both as tools (don't forget to add the ATP to your ligations) and as the very objects of study; what does the recently discovered "homeoprotein" actually *do*? Yet we shy from the gory biochemical details. Why should this be so? Is it because of a lingering distaste for the blood and guts origin of the subject? Or because our algebra stopped short of the Michaelis–Menten equation, which we were led to think lay at the heart of the subject? Or because the chemical formulae were too big to remember, and the pathways too complex to comprehend except by rote the night before the exam? Or because explanations too often stopped before they really made sense? This is a topic that needs exploring at greater length, precisely because it seems to cause such trouble. de Duve includes some appendices dealing with biochemistry and bioenergetics which would have been helpful in the body of the text, but they are at the end of the second volume so consulting them is not encouraged. Probably you either have to grasp the nettle and give the full treatment, or leave it out altogether.

In the nucleus we are for the most part in conceptual country which de Duve understands very well, but doesn't know like he knows his organelles. de Duve is not alone in speaking of "the nucleus" as standing for almost all of molecular biology; this is common parlance among cell biologists. Yet molecular biology as I understand it has very little to do with the nucleus as such. It stands for a much more highly conceptualized scheme of things that transcends (for the time being) its cellular location. Actually, we don't even understand clearly why there is such a thing as a nucleus; bacteria get along without it and *Saccharomyces cerevisiae* commits mitosis without ever losing its nuclear membrane. Curiously, this kind of issue is not considered here. There is discussion of what the nuclear membrane lets in and out, of nuclear pores and how little we understand of how they work, of how the membrane vesiculates prior to mitosis (we have to wait for this to happen to get out!), but not of why it's there in the first place. (Alberts *et al.* in *Molecular Biology of the Cell* suggest that it's to keep protein synthesis out, thus allowing splicing to occur.) I was disappointed by de Duve's silence on the matter, for elsewhere he almost always has something interesting to say.

These two volumes are an extravagant buy for the individual, but they must go into my college library and I would advise intending biologists who have not done the subject at school to try and get hold of them before they go up to university. For, despite their deficiencies, the books are a splendid introduction to cell biology, and the first hundred pages could and probably should be read with profit and enjoyment by every biologist. □



From *A Guided Tour of the Living Cell*

Stopped in time — rows of cilia on the surface of Paramecium tetraurelia *arrested in the middle of their synchronous beat.*

Tim Hunt is a Lecturer in the Department of Biochemistry at the University of Cambridge.

Nature magnified

Peter Evennett

Single Lens: The Story of the Simple Microscope.
By Brian J. Ford.
Heinemann/Harper & Row: 1985.
Pp. 182. £10.95, $14.95.

THE simple microscope, one whose optical system consists of a single lens, operating in the manner of a magnifying glass, has a distinguished place in history and quite remarkable capabilities, little known to the general scientist. The origins of its use are obscure, but by far the best known early work is that of Antony van Leeuwenhoek of Delft, communicated in the form of a series of letters to the Royal Society from 1673 until shortly before his death in 1723. One third of Brian Ford's book is devoted to Leeuwenhoek, his microscopes and specimens.

Using single-lens microscopes, Leeuwenhoek was the first to observe Protozoa, bacteria and spermatozoa: truly microscopic objects. In contrast Robert Hooke, Leeuwenhoek's contemporary working in London who published *Micrographia* in 1665, used a compound microscope (consisting of two lenses, objective and eyepiece). Both men's observations were excellent within their own limits, but while Hooke's work — on relatively large objects such as the flea or the sting of the nettle, for example — extended existing knowledge only slightly (though illustrating it most beautifully), Leeuwenhoek broke entirely new ground. The reason for this was principally his choice of microscope: lens design was then in its infancy and, to put it crudely, one poor lens gave better results than two poor lenses.

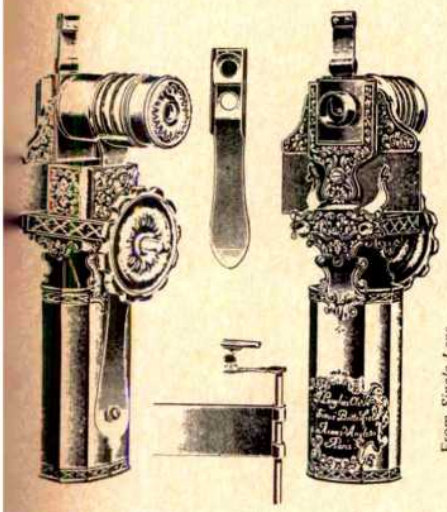
In addition to his letters, Leeuwenhoek also sent specimens, in small paper packets pasted to the letters; many of these survive in the archives of the Royal Society. In researching this book, Ford took some of the specimens to Utrecht and photographed them with an original microscope, demonstrating to those of us unlikely ever to lay hands on one the nature and quality of the image of a Leeuwenhoek microscope. A study of the surviving instruments shows that they were capable of resolving features as small as one micrometre, with a magnification of several hundred times — a performance as good as that of many school microscopes of today; indeed, for 300 years single-lens microscopes have been

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apable of revealing most of the significant tructures that can be resolved by a modern ight microscope.

After Hooke and Leeuwenhoek, the ompound microscope increased in opularity as a "drawing-room" instrunent, presumably because of its impressive ppearance and greater ease of use; Ford lescribes the progress of the simple nicroscope in the form of the cheap flea-glass, the more versatile compass microcope, and the screw-barrel and later lesigns. A simple microscope is little more



From *Single Lens*

Art of the instrument-maker — an elaborately decorated simple microscope produced in Paris, c.1720.

han a device to hold a specimen and a lens n suitable relationship one to the other, its lesign naturally influenced by the nature of the specimen to be examined. Around he middle of the eighteenth century the 'aquatic" microscope was designed, arranged to stand on a table, with a movable ens to enable it to scan sensitive creatures uch as *Hydra* without disturbance. Linnaeus owned one of these, and a similar hough superior example was used by the Scottish botanist Robert Brown in 1827 when he discovered "active molecules" now known as Brownian movement), and a few years later when he described the nucleus and cytoplasmic streaming in the plant cell. Darwin took a microscope of this kind on the *Beagle* expedition in 1831, on Brown's advice, and wrote of his purchase of another in 1848. An essentially similar nstrument is still available as a "dissecting nicroscope".

The simple microscope has not been well documented previously. Brian Ford's book s a clearly written non-technical account of the instruments, the lenses, their performance and use, illustrated with historical necdotes, quotations and drawings, and with a useful bibliography. It will interest anyone who uses a microscope and many others besides. □

Peter Evennett is a Lecturer in the Department of Pure and Applied Zoology, University of Leeds, and Editor of the Proceedings of the Royal Microscopical Society.

Psychiatry: beyond analysis

Anthony W. Clare

The Broken Brain: The Biological Revolution in Psychiatry.
By Nancy C. Andreasen.
Harper & Row: 1985. Pp.278. $16.95, £12.95.

A COMMONLY expressed dictum, usually by psychiatrists, is that there has been a revolution in psychiatry. Back in 1966, the late Sir Aubrey Lewis of the Maudsley Hospital wryly noted the common and invigorating habit of psychiatrists to rejoice "that they live in an age of rapid and impressive advance". Advances there have surely been but whether any of them warrants the description "revolutionary" is highly questionable.

Just after the Second World War, the new antipsychotic phenothiazines and the tricyclic antidepressants were hailed by psychiatrists such as William Sargant in Britain and Nathan Kline in the United States as the harbingers of a revolution in physical treatment which would empty the mental hospitals and re-establish psychiatry within the mainstream of general medicine. Even before the introduction of these drugs, however, the mental hospital population on both sides of the Atlantic had begun to fall and this trend too was acclaimed as reflecting a revolution in care. Much of its momentum derived from the realization that institutionalization of the chronic mentally ill could in many instances worsen the condition and add substantial secondary impairment. A vigorous new emphasis within psychiatry was born, social psychiatry, whose enthusiastic adherents emphasized the importance of energetic rehabilitation and rapid reintegration of the mentally ill within the general community. In the United States, the presidential involvement of John F. Kennedy gave the movement a particular momentum such that over a quarter of a century the mental hospital population has fallen by nearly 75 per cent (compared with the more pedestrian British decline of 25 per cent) and a whole chain of community mental health centres has been deployed across the continent.

The mid-1960s, a turbulent time within as well as outside psychiatry, witnessed the flowering and then the demise of arguably the most revolutionary idea of all, namely that mental illness does not actually exist but is a myth, a metaphor or merely an obfuscating and remarkably sticky label. The mentally ill were variously portrayed as outsiders, deviants and voyagers on a perilous journey of self-discovery and transcendentalism. Around the same time, the more prosaic yet more enduring advances in behavioural theory in turn yielded a series of promising techniques of behavioural treatment, including desensitization, operant conditioning and flooding, offering hope to sufferers of the most intractable of neurotic conditions such as phobic anxiety and obsessive compulsive disorder.

If one uses the term "revolution" in the sense of the undermining and overthrow of the established order, then none of these developments seriously threatened psychiatric orthodoxy. Psychotropic drugs, psychiatric rehabilitation, the development of community-based facilities, the application of behavioural techniques — each has been comfortably absorbed within the general mainstream of psychiatry. In the process, the original enthusiasm has been tempered by time and by bitter political experience. In the case of the much-vaunted community mental health centre programme, for example, professionals, politicians and the public appear deeply divided not about whether it was a good or a bad idea but about who precisely is responsible for the disaster that it has turned out to be. Its critics blame the haste, the ill-conceived design which has led to the warehousing of large numbers of chronically ill psychiatric patients in squalid and anti-therapeutic conditions within a community which clearly does not care, and the failure of its advocates to acknowledge the degree of disability manifested by those who are most seriously mentally ill. Its supporters attack the failure of Federal government to honour funding pledges made by its predecessors and the marked ideological shift in the way that Washington regards the concept of community welfare.

Unabashed, American psychiatrists are now acclaiming a new revolution. The author of *The Broken Brain*, a book revealingly subtitled *The Biological Revolution in Psychiatry*, is moved to declare with the enthusiasm of one who has witnessed the fall of some psychiatric Bastille, that "Psychiatry, like the prodigal son, has returned home to its place as a specialty within the field of medicine". For Nancy Andreasen, Professor of Psychiatry at Iowa College of Medicine, psychiatry is becoming increasingly scientific and biological in its orientation and medical science is now convinced that the serious forms of mental illness, such as schizophrenia and manic-depression, are due mainly to abnormalities in brain structure and chemistry.

British readers, familiar with the relatively close relationship between psychiatry and general medicine in this part of the world, may wonder what all the fuss is about and where the American prodigal son has been all these years. Yet the argument that in the United States at least there may indeed be a significant shift in psychiatric thinking, if not a revolution, does merit closer examination. There was a time, less than 25 years ago in fact, when it was difficult if not impossible to become the chairman of an American department of psychiatry unless one had undergone psychoanalytical train-

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QUATERNARY DATING METHODS

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ing and a personal analysis. Up to quite recently, most major medical schools accepted psychoanalytically orientated psychotherapy as the foundation of psychiatric theory and practice, and in a number of them — most notably New York Medical College, Columbia, Pittsburgh, Tulane, Western Reserve and North Carolina — actual postgraduate psychoanalytical institutes were established. Not merely were most departmental heads psychoanalysts, so were most of the members of their departments, their psychiatric residents, their own personal psychotherapists, their wives and many of their government site visitors. In psychosomatic medicine, child and adolescent psychiatry, the psychotherapy of the neuroses, social casework, individual and group therapy, the impact and influence of psychoanalytic theory and practice were enormous.

Yet even at its zenith psychoanalysis was under fire. In 1959, two research psychiatrists, J. MacIver and F. Redlich, writing in the *Journal of the American Medical Association*, critically observed that "the hard truth is that we are expending enormous effort, time and money to train a highly specialized group of experts who will devote the bulk of their professional careers to a small group of highly selected patients". Several years later, a distinguished biologist, Seymour Kety, was appointed to head the psychiatric department at Johns Hopkins. Together with David Rosenthal and Paul Wender, he embarked on a programme of research which was to cast light on the chemical nature of synaptic transmission, develop a number of potent drugs capable of specific actions against many of the symptoms of severe mental illness, and provide new and substantial evidence of genetic factors operating in the aetiology of the major psychoses. Indeed one prominent psychoanalyst spoke prophetically at the time of Kety's appointment that it was calculated "to drive another nail into the coffin of analysis".

Despite these and related biological developments, however, American psychiatry clung on to its heavily analytical reputation until the past few years when suddenly it appears to be discarding it rapidly. Professor Andreasen expresses the widely held view that it is the exciting developments in neuroanatomy, neurochemistry, neuropathology, neuropharmacology, genetics and behavioural neurology which have not just undermined the preeminence of psychoanalysis but significantly eroded it. Her book, in a highly readable if occasionally simplistic fashion, emphasizes the manner in which such explanatory ideas as the catecholamine theory of depression and the dopamine theory of schizophrenia, while partial, flawed and even contradictory, have in their own way reminded a whole new generation of young psychiatrists of the possibilities and challenges inherent in the biological approach. Hemispheric localization, limbic system function, GABA, positron-emission tomographic scanning, the dexamethasone suppression test, benzodiazepine receptors — the topics included and discussed, while in no way adding up to a coherent and plausible theory of brain dysfunction in psychiatric disease, illustrate the potential for research and the bewildering number of growing points.

In her enthusiasm for these developments, Andreasen, in common with many of the new biologists, ignores the lessons of recent history. The reverses suffered in turn by psychoanalysis and social psychiatry, each originally hailed as true revolutionary breakthroughs in the way we understand and treat mental illness, should not be ignored. If psychiatry in the United States has a fault it surely is its tendency to suffer from what Gore Vidal, in another capacity, has termed the peculiarly American affliction of "historical amnesia". At times, Professor Andreasen acknowledges the limitation of reducing all psychopathology to the level of abnormally firing neurones, all psychiatric disorder to the level of brain disease. On other occasions, her zeal gets the better of her and she commits precisely that error. Yet psychiatry has been here before, just over a century ago, but one will look in vain in the index for the names of Griesinger and Maudsley, Westphal and Meynert, men who also nourished the hope that one day severe psychopathology might be reduced to a matter of disturbed cerebral function.

The resurgence of biological enthusiasm doubtless owes much to the new and exciting plethora of scientific tools and techniques applicable to unravelling the complexity of brain function. It owes much too to the realization that psychoanalytic theory is sterile and psychoanalytic treatment is ineffective. But before participating in a single-minded and headlong rush to the brain, enthusiasts would do well to heed Kety's reminder that the language that we speak, the principles we value, the thoughts we think, the decisions that motivate our daily actions are to a greater extent a reflection of what we have learned than the result of how we are constituted. "The differences among us in respect of these qualities", he has recently written, "are not to be found in the structure or chemical composition of our brains but lie largely within the scope of the psychological and social sciences". If this is ignored, then crude biological reductionism is the result and the possibility that psychiatry might act as the bridge in medicine between the biological sciences on the one hand and the psychological and social sciences on the other hand evaporates. That is hardly a development worthy to be considered revolutionary. □

Anthony W. Clare is Professor in the Department of Psychological Medicine at St Bartholomew's Hospital Medical College, University of London.

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Sowing the seeds of awareness

David Streeter

Green Inheritance.
By Anthony Huxley.
Collins–Harvill/Anchor–Doubleday: 1985. Pp. 193. £9.95, $19.95.
Our Green and Living World: The Wisdom to Save It.
By Edward S. Ayensu, Vernon H. Heywood, Grenville L. Lucas and Robert A. Defilipps.
Cambridge University Press/Smithsonian Institution Press: 1984. Pp. 255. £12.95, $24.95.
In the Rain Forest.
By Catherine Caufield.
Heinemann/Knopf: 1985. Pp. 304. £10.95, $16.96.

ONE of the most striking features of current radio and television, in Britain at least, is the number of natural history programmes that are finding their way into the schedules. Radio 4 alone, for example, is currently putting out five such programmes a week. Until recently, however, wildlife was considered to consist wholly of animals; even in David Attenborough's celebrated television series *Life on Earth*, plants made a significant appearance in only one out of the thirteen parts.

David Bellamy's early programmes have done much to redress the balance and coincided with the emergence of a higher profile conservation movement drawing attention to, among other issues, the attrition of the tropical rain forests. The established conservation bodies began to take notice. In 1980 the Fauna Preservation Society symbolically changed its name to the Fauna and Flora Preservation Society, and in March 1984 the World Wildlife Fund and the International Union for the Conservation of Nature and Natural Resources launched an international programme for plant conservation. As part of this campaign, two books, both aimed primarily at the general market, have appeared almost simultaneously. *Green Inheritance* is published as the official book of the World Wildlife Fund's Plants Campaign and is written by Anthony Huxley, a well-known writer and lecturer on plants. *Our Green and Living World* has an appropriately international authorship of an African, two Europeans and an American.

The purpose of both books is to arouse concern over the quite staggering rate of destruction of much of the Earth's natural vegetation and to point to the consequences. Inevitably one draws comparisons between the two. As a plea for plants the case put by Anthony Huxley is the more compelling. The factual material is well-researched, and while there are lapses here and there when the subject becomes more physiological the text as a whole provides a fascinating and lucid account of mankind's relations with and dependence upon plants. Separate chapters deal with the role of plants as food, fuel, medicine and objects of beauty, as well as with the wider role of vegetation in atmospheric and soil processes. Accounts of the origins and spread of crop plants and the medicinal uses of plants are particularly good, the latter containing some quite astonishing information such as that on the uses or, more properly, abuses of the thornapple. Apart from Huxley's persuasive style, much of the success of *Green Inheritance* rests with its design. The combination of a continuous discursive text alongside short illustrated vignettes providing more detailed information works well, and the standard of graphics and art work is high.

Our Green and Living World covers much the same ground and overall, perhaps, is the more authoritative of the two. However, for the general reader it is likely that its message will be less compelling, the style sometimes being closer to that of an undergraduate text. Unlike *Green Inheritance* it includes eight chapters on different vegetation types from deserts to rain forests and from wetlands to oceans. It is also more comprehensive on the subjects of genetic conservation and under-exploited crops (the separate chapters on firewood and cellulose are particularly good). Like *Green Inheritance*, the production can only be described as lavish, some of the photographs being quite superb.

The overall lesson of the two books is clear, and in particular both focus attention on the devastation of the tropical rain forests. Five years ago the US National Academy of Sciences calculated that over 50 million acres were destroyed or seriously damaged each year and the UN's Food and Agricultural Organization estimated in 1981 that at present rates almost one-fifth of the world's remaining rain forest would have gone by the end of the century. Catherine Caufield's *In the Rain Forest* is a carefully researched collection of essays on various aspects of rain forest ecology, exploitation and destruction. Subjects range from the building of the town and dam of Tucurui in the Amazon basin to the discovery of quinine. Figures and statistics spill off the pages, and the value of the book is enhanced by good indexing, text notes and a comprehensive bibliography, but it is not — and does not pretend to be — a textbook on rain forest ecology. For anyone unfamiliar with the rain forest this is undoubtedly a good read and an intentionally perturbing one. □

David Streeter is a Reader in the School of Biological Sciences, University of Sussex.

Who cares?

Marian Dawkins

Strategies of Being Female: Animal Patterns, Human Choices.
By Evelyn Shaw and Joan Darling.
Harvester/Walker: 1985. Pp. 164. £12.95, $14.95.

IT WAS only in the last few pages of this book that I understood what the authors' purpose in writing it really was. Initially, I had thought it was going to be yet another polemic about the allegedly male-dominated sexist views of sociobiology in general and its treatment of sexual behaviour in particular. As I have never been able to understand this particular criticism of sociobiology (sociobiological emphasis on the evolutionary importance of female choice seeming, if anything, to overemphasize the role of females), I began the book by being somewhat out of sympathy with the authors. Some distinctly group selectionist phraseology — such as "reproducing in anticipation of the species' future" (p.32) and "the true biological reasons for having children [are] to assure the survival of the human species" (p.121) — only increased my antipathy.

But I was curious. How were the rampaging, promiscuous, initiative-taking females they cite from all corners of the animal kingdom going to be related to our own species, as promised in the introduction? Would promiscuous female fish or birds be used as a model for human beings? Would female hyenas, with their sham male genitalia, be held up as an example to us all?

Only in the last two chapters do Shaw and Darling argue that human females are in fact different from those of any other species. Females of other species may show a wide variety of sexual behaviour, but only human females can choose which particular one they will adopt for themselves. Looking after children is not, they argue, "biologically programmed in the genes of the female". "Words — not our hormones, and not some neurological Gestalt etched into our brains — teach us how to find a mate, who is appropriate as a mate and who isn't, when to have babies, how to give birth to them, and how to take care of them" (p.152). It is all the fault of culture, and the "myth of maternalism" in particular, that women are the ones most commonly adopting a child-caring role.

It is to attack this myth of maternalism — the claim that mothers are inherently better at caring for offspring than fathers — that turns out to be the book's main purpose. Men, it is argued, are just as good at looking after children as women are, if only they are given the chance. The trouble is they are so rarely given the chance. In a wide variety of cultures, women exclude men and refuse to allow them to be present at the birth of their own children. Shaw and

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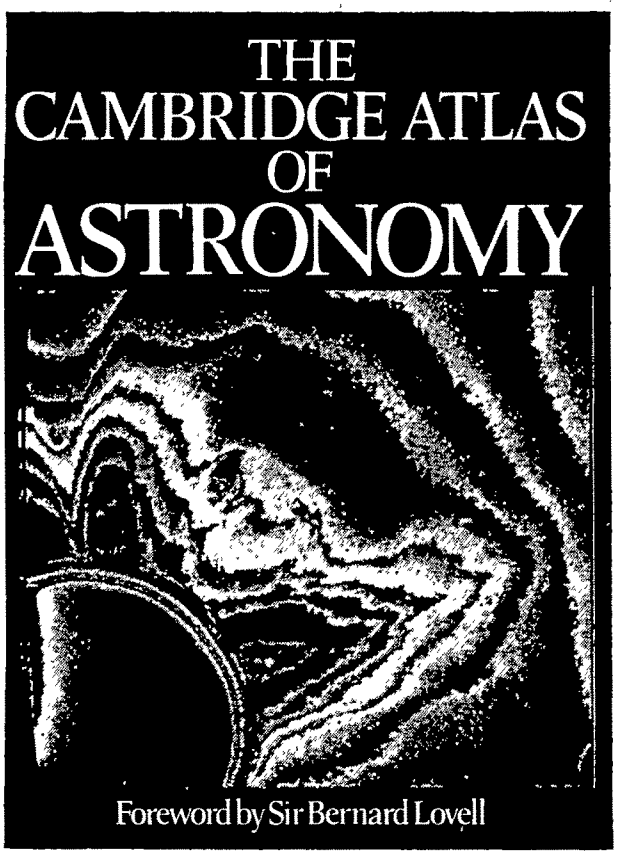
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Darling argue that if only men could be present at the birth, they too would form strong bonds with their children and take on the care-taking role usually associated with mothers.

In support of this idea, they cite male wild-dogs, male marmosets and other species in which males take an active part in looking after the young. They also quote Rosenblatt's finding that male rats will



Kevin Carlson/Ardea.

Mother love — black-faced vervet monkey with infant.

begin to lick and retrieve young after they have been exposed to them for several days. The male's tendency to care for young is latent and revealed only if given the opportunity: "And just like a father rat, the human father is denied access to the environmental cues that would turn on his 'maternal' behaviour" (p.148).

There may or may not turn out to be something in all this. It is an interesting hypothesis, one which is certainly worth thinking about. What is disturbing to me is the way it is put over. Perhaps this is simply because the book is written in a popular style. Maybe words such as "babes" and "critters" have to be used to make sure readers do not feel overwhelmed by technical jargon; maybe a case has to be overstated in order to attract a public who would be bored by too much weighing up of evidence. But do we have to swing from male sexism to an equally unattractive female sexism? If we accept that women may wish and be able to fulfil a wider variety of roles than they often do, do we have to see society purely in terms of womens' choices and womens' destinies?

Shaw and Darling emphasize that "female" does not have to mean passive, meek or monogamous. But at the same time, perhaps unintentionally, they make female wishes and female roles dominant, without explaining why this kind of sexism is any more convincing — or any more acceptable — than the male kind. □

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Ways to get back to the track

David Pimentel

State of the World 1985: A Worldwatch Institute Report on Progress Toward a Sustainable Society.
Project director Lester R. Brown.
W. W. Norton: 1985. Pp.301. Hbk $22.75, £18.95; pbk $9.50, £7.95.

IN PUBLISHING this detailed analysis of "the state of the world", the Worldwatch Institute has performed a valuable service for the public, government and industry. Examined in it are the interdependence of human population, global environment and world economy. Specifically, the authors assess the effect of population growth on deforestation, overgrazing, soil erosion, atmospheric pollution, and water and energy shortages. With this as a basis, they go on to consider the influence of natural resource degradation and resource shortages on the economy and standard of living of society.

Early in the book Brown and his colleagues tackle the question of whether climate change in some regions of the Earth may be population induced. They suggest that, indirectly, population growth may reduce rainfall because of overgrazing and other activities that remove or reduce vegetation cover. The increased exposure of bare soil increases the albedo: "Where this happens, as on the fringes of the Sahara, the affected areas reflect more heat into space". Canadian meteorologist, Kenneth Hare, who has investigated desertification in Africa, is quoted as concluding that "we seem to have arrived at a critical moment in the history of mankind's relation to climate. For the first time we may be on the threshold of man-induced climatic change". If indeed water shortages are population induced, then the decline can be reversed in some regions only by massive programmes in family planning, tree planting, soil and water conservation, and ecological–agricultural development on a huge scale.

The authors acknowledge the fact that major efforts to produce more food per hectare of land have slowed the growth of hunger, noting that agriculturalists have made use of biological diversity to breed new crop varieties that are more efficient in converting water and fertilizers into grain and other foods. At the same time, farmers have increased the amount of land cultivated and have greatly expanded the use of irrigation and fertilizers. Irrigation, for instance, has grown nearly three-fold since 1950 and fertilizer usage about nine-fold during much the same period. All these strategies have increased crop yields.

It remains to be seen, however, how far agricultural technology can go in producing more food from the world's limited arable land resources. We already know that there are certain physical and chemical constraints on the capacity of plants to capture and fix solar energy into biomass. Recognizing such limitations, the authors question whether biotechnology will make the major changes necessary to continue improving yields. A major concern to emerge here is the effect that the rapid loss of biological diversity will have on the success of biotechnology and genetic engineering, but in placing emphasis on this point the authors neglect to explain that *most* species in nature are vital to the normal functioning of the ecosystem. For example, agriculture cannot function successfully with only its crop and livestock species; many organisms, especially the small organisms (such as microbes and insects) are also essential. Thus on a hectare of good land with ample water, the small organisms outweigh human biomass by at least 200 times and carry out the vital tasks of degrading wastes and chemical pollutants, and of recycling other resources.

In a chapter on energy efficiency, William Chandler reports on the important role that fossil energy plays in producing food, purifying water, protecting us from numerous kinds of diseases and transporting resources from one region to another. Several sound arguments support the view that conservation of energy and improved energy efficiency are necessary for economic improvement in both developing and developed countries. But although fossil energy is essential to our present standard of living, almost everyone agrees that new sources of energy are needed, preferably renewable in form. However, the chapter entitled "Harnessing Renewable Energy" is rather disappointing. Several of the sources cited lack scientific credibility. For example, one paper quoted here reports that producing ethanol from corn grain provides a net energy return. This is not so; in fact, the net energy loss is about 30 per cent greater than the ethanol energy produced.

Also highlighted is the idea of shifting from the corn–soybean crop mix now typical in the mid-west of the United States to a corn–sugar-beet mix. This proposed plan, which is supposed to supply all food needs for livestock plus up to 700 million barrels of ethanol, has numerous technological and agricultural flaws and has been

Conservation in Britain

In association with the publisher Webb & Bower, and sponsored by Gulf Oil, the Conservation Foundation have published *The Conservation Review*, their third annual report for "everyone concerned with preserving our heritage and natural environment".

The *Review* is intended to increase public awareness of environmental issues and draw attention to the activities of various organizations, local, national and international. Among the contributors are David Bellamy, Patrick Jenkin, Tony Soper and Jean Medawar. Price is hbk £10.95, pbk £4.95.

discounted by most energy specialists. Further, nowhere do the authors acknowledge the environmental risks associated with certain renewable systems. For example, the widespread burning of crop residues and dung as a fuel is severely intensifying soil erosion and water run-off, and is robbing the soil of essential nutrients. Because soil erosion and water run-off are a major feature of the *Report*, it is especially unfortunate that biomass energy systems are not identified as major causes of these problems. There are also drawbacks to hydropower, but again no mention is made of them. Dams and reservoirs have to be constructed to run hydropower systems, and in the United States, for instance, about 13,000 hectares of productive agricultural land has to be flooded per 1×10^9 kWh/yr of electricity produced (sufficient electricity for a city of 100,000). This is not to imply that hydropower is not a valuable energy system in the United States and many regions of the world, but that environmental risks should be evaluated to determine if the benefits for a region outweigh the costs.

One of the most challenging aspects of the resource and environmental problems facing us today is their "gradual, insidious nature". For example, a single rain storm may erode 1 mm of soil weighing 15 tonnes from a hectare. That loss may be imperceptible, but it does not take many rain storms and many years to remove the 2.5 cm of topsoil that generally requires 200 to 700 years to replace. As Brown and Wolf point out, "few countries will succeed in attempts to boost domestic food at the rate demanded by population growth if soil-depleting agriculture continues".

The authors of the *Report* clearly explain that soil, water, energy and biological resources are being threatened not only by the rapidly growing population but also by poor management practices in agriculture and other industries. Further, many excellent plans are suggested for tackling the problems. But, as they caution, long-term solutions will require the efforts of individual intellects and of politicians to get society back to the track — the intellects to identify the direction for change, and political leadership to devise and carry out the appropriate policies for stabilizing populations and effectively managing environmental resources.

State of the World is a thorough and up-to-date examination of the influence of mankind on the Earth's various resources. Particularly helpful and timely are the solutions to the complex problems that are identified in the book. This is an exceptionally well-written report which scientists and industrial and political leaders would do well to read and to act upon. □

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Biology as science

A.J. Cain

The Cambridge Encyclopedia of Life Sciences.
General editors Adrian Friday and David S. Ingram.
Cambridge University Press: 1985. Pp.432. £25, $45. To be published on 9 May.

TO TRY to encompass within a volume of 432 pages a complete encyclopaedia of the life sciences strikes one as a most noble aim and a forlorn hope. The editors and contributors have succeeded in this book to a really remarkable degree.

The volume is divided into three parts: "Processes and Organisation" (the cell, the organism, genetics, behaviour and sociobiology, ecology); "Environments" (marine, coastal, terrestrial, freshwater and wetland, living organisms as environments); and "Evolution and the Fossil Record" (the evolutionary process, palaeontology, early events in evolution, origin and development of the land flora and fauna, recent history of the flora and fauna). A classification of living organisms, a species index (generic also) and a subject index complete the book.

The contents page refers under Part 2 to what appears to be an unnumbered chapter on biogeographical zones. The relevant pages in fact carry a set of maps only, and neither biogeography nor any names of biogeographical regions appear in the subject index. There are, however, excellent accounts of plate tectonics, numerous maps of the drifting continents, and much information on regional history and animal diversity in the section on the origin and development of the land flora and fauna, the best place to discuss the development of biogeographical diversity.

The book is written in a very readable style and has obviously been most carefully corrected in proof — one of the very few books I have seen in recent years that can be put into the hands of the young without a warning against bad spelling and grammar. It is a model of exposition, excellently illustrated. In the words of the editors:

The Cambridge Encyclopedia of Life Sciences is not a natural history cataloguing the living world, it does not attempt to argue a case for or against biological conservation, nor does it consider the yet greater ethical problems which arise from the genetic manipulation of cells. Instead, the *Encyclopedia* seeks to present biology as a science, and in addition constitutes a plea for considering all biological phenomena as indissolubly linked, a circumstance which underlies both the frustration and the fascination felt by all life scientists.

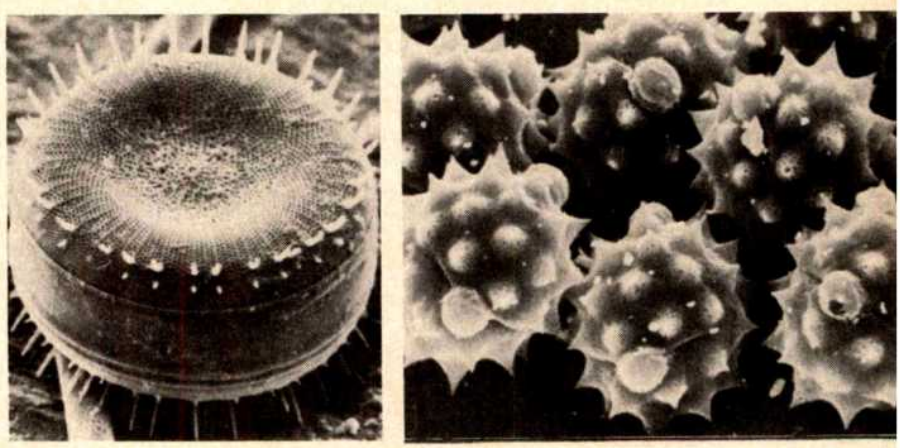
Inevitably, the necessary compression means that many topics can only be treated summarily, as a series of key concepts, their interrelations, and a brief mention of their empirical basis. In some ways this is a positive advantage, forcing the writers to get to the real bones of the subject. One could give a series of lectures on the single paragraph (p.296):

Some biologists have argued that if we can reconstruct the pattern of evolution, the *phylogeny*, this will provide insight into the ways in which evolutionary change occurs. The opposite point of view is that knowledge of the processes of evolutionary change is essential before it is possible to find out the pattern of relationships between organisms.

Yes, indeed!

Equally inevitably, specialists will be dissatisfied because so much can only be mentioned. It would take a team of reviewers as large as the team of authors and editors (31, 22 of them in Cambridge) to deal with the chapters fully. In my own speciality, I notice only the total absence of ring species and allopolyploid plants from the section entitled "Mechanisms of Speciation", in which too much space is given to the latest fads in speciation theory. As against that, what a pleasure it is to see a proper discussion of parasites in the section on living organisms as environments. Surely there are more species of parasitic than of free-living organisms, yet this major aspect of evolution and the ecosystems of the world is usually mentioned merely in passing except in highly specialized texts. □

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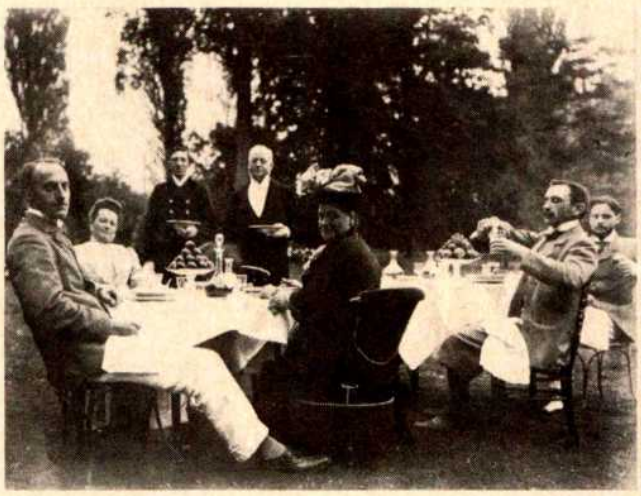
Revelations of the scanning electron microscope — the diatom Stephanodiscus *(left) and pollen of* Chrysanthemum mycoais.

A matter of taste

Nicholas Kurti

On Food and Cooking: The Science and Lore of the Kitchen.
By Harold McGee.
Charles Scribner's Sons: 1984. Pp.684. $29.95.

How refreshing it is to be treated to an inspiring blend of botany, zoology, physiology, anatomy, biochemistry, and, of course, gastronomy. In his introduction to what is almost a scientific, literary and historical encyclopaedia of food and its enjoyment, Dr McGee admits to the importance of experience, intuition and a discriminating palate in preparing food, but he also asserts that "a general understanding of what is going on in the pan ... can compensate for a lack of familiarity with particular ingredients and techniques". The book is studded with examples of the beneficial intrusion of science into the kitchen, often expressed in vivid terms, such as the remark that "roast and meringue are different outcomes of the same process [but] the knowledge of protein coagulation need not diminish the pleasure in consuming a well-made roast or a meringue". McGee also reminds us that "while we may be up on Black Holes and the Big Bang and the Primordial Soup we can't explain how flour thickens soup".



Mary Evans

Nearly four-fifths of the book is devoted to accounts of the foodstuffs that go into our diet, grouped according to their origin rather than their chemistry. Scientific explanations are interspersed with historical remarks and tips on how to avoid or rectify cooking disasters and there is no lack of debunking (or justifying, as the case may be) of culinary and dietary fads. For example the much-vaunted blessings of fibre and bran are discussed critically but with tolerance. The author recognizes the nutritional value of whole-grain bread, but also quotes the rickets epidemic in Dublin during the Second World War when rationing of dairy products led to a low calcium intake, the assimilation of which was inhibited by the high bran content of the diet.

In discussing the progress of meat from the slaughterhouse to the table, McGee emphasizes the need to keep the animal free of stress before slaughter; muscular tension depletes the glycogen supply, less lactic acid accumulates in the muscle after slaughter and the result is inferior "dark-cutting" meat. The use of tenderizers based on naturally occurring proteolytic enzymes is also described but, surprisingly, the use of the hypodermic syringe to obtain even distribution of the tenderizer is not mentioned. And although it is true that these enzymes work faster at higher temperatures, I have found that fresh pineapple juice will tenderize pork chops at room temperature in 2-4 hours.

There is too an excellent explanation of the conflicting demands on the *rôtisseur* trying to achieve the conversion of the collagen in connective tissues into gelatin (a slow process) without drying out and toughening the muscle fibres. There is, however, nothing about the low-temperature (less than 75°C) roasting technique pioneered by Sir Benjamin Thompson, Count Rumford (1753-1814), the Anglo-American soldier, statesman and natural philosopher who was also a culinary innovator. This method works well: I proved its excellence to my own satisfaction — and, apparently, to the satisfaction of those who ate the joint — by roasting a 4lb leg of lamb for 8 hours in a slow oven.

McGee gives a full account of the arguments about the use of copper bowls when making meringues, culminating in a re-run of the beautiful piece of work undertaken at Stanford University by McGee and his colleagues (*Nature* **308**, 667; 1984) which showed conclusively that traces of copper ions do indeed make egg-white foam more durable through the formation of a copper-protein complex. It is a pity that neither this book nor the original article indicates the minimum amount of copper needed per egg. It is bound to be very small and phials of "meringue-improvers" could be sold at a price many thousand times the value of their contents. (I found it odd, incidentally, that albumen coagulation (which occurs at around 60°C) is regarded as an essential feature of meringues; it is well known that meringues can be made at considerably lower temperatures, even at 20°C, especially if reduced pressure is used.) There are many other useful tips for the amateur confectioner; here at last, for instance, we have a clear explanation of how peppermint cream gets into the thin crisp chocolate of after-dinner mints.

It is in the chapter on sauces that the enthusiasm of the gastronome, the artistic skill of the cook and the critical approach of the scientist shine most brightly. This is a veritable *saucier*'s vade-mecum. All the traditional tricks are explained, new techniques are suggested and there is also, among other good things, a full account of the saga of the Resurrection of a Curdled Sauce Béarnaise which began in 1977 with an article in *Nature* (**270**, 572) by C.M. Perram, C. Nicolau and J.W. Perram.

More prosaically, in writing about food additives McGee reminds those with a taste for nostalgia of "bread mixed up with chalk, alum and bone ashes", of "greens boiled with brass half-pence" and "butter manufactured with candlegrease and kitchen stuff", practices which were prevalent in mid and late eighteenth century England. Smollet bemoaned the fact that "these enormities might be remedied, but the wise patriots of London have taken into their heads that all regulation is inconsistent with liberty". We are better off now, inasmuch as food adulteration on the scale of the past can no longer be perpetrated with impunity, and the author illustrates, with the help of the cases of saccharin and sodium nitrite, the difficulties in arriving, on the basis of often incomplete scientific evidence, at solutions which satisfy public demand and reduce the risk to public health. Similar good sense characterizes the two brief sections "Food and the Body" and "The Principles of Cooking", though I was surprised that nowhere in the book are calories mentioned. I should liked to have seen at least a modest table giving the values of the principal foods. This omission may have been deliberate, however, the author wanting to emphasize in this way a view, latent throughout the text and expressed felicitously at the end of the chapter on nutrition, that "Nutrition by the numbers is no guarantee of the good life and probably precludes it by overriding the calls of appetite and pleasure".

Dr McGee's book brings to mind a remark of Count Rumford:

> The advantage that would result from an application of the late brilliant discoveries in philosophical chemistry, and other branches of natural philosophy of mechanics, to the improvement of the art of cookery, are so evident and so very important that I cannot help flattering myself that we shall soon see some enlightened and liberal-minded person of the profession take up the matter in earnest, and give it a thoroughly *scientific* investigation.
>
> In what art or science could improvements be made that would more powerfully contribute to increase the comforts and enjoyments of mankind.

Two centuries later, with the publication of *On Food and Cooking*, gastronomes and scientists alike can rejoice that Rumford's hopes are on their way to fulfilment. □

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Mathematics

Clifford Algebra to Geometric Calculus: A Unified Language for Mathematics and Physics. By DAVID HESTENES and GARRET SOBCZYK. *Reidel: 1984. Pp.314. ISBN 90-277-1673-0. Np.*

Incline Algebra and Applications. By Z-Q CAO, K.H. KIM and F.W. ROUSH. *Ellis Horwood: 1984. Pp.165. ISBN 0-85312-765-4. £16.50.*

Mathematical Aspects of Superspace. H.J. SEIFERT, C.J.S. CLARKE and A. ROSENBLUM (eds). *Reidel: 1984. Pp.214. ISBN 90-277-1805-9. Np.*

W.G. Cochran's Impact on Statistics. PODURI S.R.S. RAO and JOSEPH SEDRANSK (eds). *Wiley: 1984. Pp.431. ISBN 0-471-09912-0. £46.50.*

Astronomy

Armchair Astronomy. By PATRICK MOORE. *Patrick Stephens, Denington Estate, Wellingborough, Northants NN8 2QD. Pp.185. ISBN 0-85059-718-8. £9.95.*

Astronomical Objects for Southern Telescopes: A Handbook for Amateur Observers. By E.J. HARTUNG. *Cambridge University Press: 1984. Pp.237. Pbk ISBN 0-521-31887-4. Pbk £8.95.*

Astronomy and Astrophysics Abstracts Vol.35/36. S. BOHME, W. FRICKE, H. HEFELE, I. HEINRICH, W. HOFMANN, D. KRAHN, V.R. MATAS, L.D. SCHMADEL and G. ZECH (eds). Author and Subject Indexes to Vols 25–34 Literature 1979–1983 prepared by I. HEINRICH and L.D. SCHMADEL. *Springer-Verlag: 1984. Pp.891. ISBN 3-540-13651-7. DM 189, $68.80.*

Atlas of the Night Sky. STORM DUNLOP (ed.). *Newnes Books: 1984. Pp.80. ISBN 0-600-35113-0. £4.95.*

Clusters and Groups of Galaxies. F. MARDIROSSIAN, G. GIURICIN and M. MEZZETTI (eds). *Reidel: 1984. Pp.659. ISBN 90-277-1772-9. Np.*

Collins Guide to Stars and Planets. By IAN RIDPATH. *Collins: 1984. Pp.384. ISBN 0-00-219071-0. £8.95.*

Comets: A Descriptive Catalog. By GARY W. KRONK. *Enslow: 1984. Pp.331. Pbk ISBN 0-89490-071-4. Pbk £15.50.*

Mathematical Astronomy in Copernicus's *de Revolutionibus*. Parts 1 & 2. By N.M. SWERDLOW and O. NEUGEBAUER. *Springer-Verlag: 1984. Part 1 pp.537, ISBN 0-387-90939-7/3-540-90939-7. Part 2 pp.711, ISBN 0-387-90939-7/3-540-90939-7. DM 238, $88.80.*

Relativistic Astrophysics and Cosmology. X. FUSTERO and E. VERDAGUER (eds). *Wiley: 1984. Pp.301. ISBN 9971-966-60-3. £32.25.*

Serendipitous Discoveries in Radio Astronomy. K. KELLERMANN and B. SHEETS (eds). *National Radio Astronomy Observatory, PO Box 2, Green Bank, WV 24944: 1984. Pp.321. Np.*

Physics

Advances in Applied Mechanics. JOHN W. HUTCHINSON and THEODORE Y. WU (eds). *Academic: 1984. Pp.376. ISBN 0-12-002024-6. $89, £67.*

Applied Atomic Collision Physics. Vol. 2 Plasmas. C.F. BARNETT and M.F.A. HARRISON (eds). *Academic: 1984. Pp.500. ISBN 0-12-478802-5. $80, £62.*

Basic Plasma Physics II. Handbook of Plasma Physics, Vol. 2. A.A. GALEEV and R.N. SUDAN (eds). *North-Holland: 1984. Pp.850. ISBN 0-444-86645-0. Dfl 495, $190.50.*

Computational Methods of Neutron Transport. By E.E. LEWIS and W.F. MILLER. *Wiley: 1984. Pp.401. ISBN 0-471-09245-2. £49.80.*

Elementary Particles. By RAMON PRASAD. *Geometrica Press, 21 Nassington Road, London NW3 2TX, UK: 1984. Pp.76. Pbk ISBN 0-904377-02-4. Pbk £5.*

Equilibrium Properties of Fluids and Fluid Mixtures. By ALEKSANDER KREGLEWSKI. *Texas A&M University Press: 1984. Pp.253. ISBN 0-89096-183-2. $32.50.*

General Relativity. By ROBERT M. WALD. *University of Chicago Press: 1984. Pp.491. Pbk ISBN 0-226-87033-2. Pbk £25.50, $30.*

The History of Classical Physics. By R.W. HOME. *Garland: 1984. Pp.324. ISBN 0-8240-90607-5. $53.00.*

Mathematical Physics Reviews. Vol. 4. S.P. NOVIKOV (ed.). *Harwood: 1984. Pp.280. ISBN 3-7186-0146-X. $99.50.*

Modern Methods of Particle Size Analysis. HOWARD G. BARTH (ed.). *Wiley: 1984. Pp.309. ISBN 0-471-87571-6. £58.50.*

Molecular Quantum Electrodynamics: An Introduction to Radiation–Molecule Interactions. By D.P. CRAIG and T. THIRUNAMACHANDRAN. *Academic: 1984. Pp.324. ISBN 0-12-195080-8. Np.*

Nuclear Tracks and Radiation Measurements: Solid State Nuclear Track Detectors. G. ESPINOSA *et al.* (eds). *Pergamon: 1984. Pp.651. ISBN 0-08-031420-1. £68.75, $110.*

Biological Sciences

Ionic Channels of Excitable Membranes. By BERTIL HILLE. *Sinaur:1984. Pp.426. ISBN 0-87893-322-0. $32.50.*

Laboratory Guide to Insect Pathogens and Parasites. By GEORGE O. POINAR and GERARD M. THOMAS. *Plenum:1984. Pp.392. ISBN 0-306-41680-8. $49.50.*

Lecture Notes on Coastal and Estuarine Studies. Vol. 8 Marine Phytoplankton and Productivity. O. HOLM-HANSEN, L. BOLIS and R. GILLES (eds). *Springer-Verlag:1984. Pp.175. Pbk ISBN 3-540-13333-X/0-387-13333-X. Pbk DM 36, $14.20.*

The Lesser Apes: Evolutionary and Behavioural Biology. HOLGER PREVSCHOFT *et al.* (eds). *Edinburgh University Press:1984. Pp.709. ISBN 0-85224-448-7. £65.*

Leukemia. E. THIEL and S. THIERFELDER. *Springer-Verlag:1984. Pp.305. ISBN 3-540-13289-9/0-387-13289-9. Np.*

Lipid Research Methodology. JON A. STORY (ed.). *Alan R. Liss:1984. Pp.296. ISBN 0-8451-1659-2. £37.*

Lipids in Plants and Microbes. By J.L. HARWOOD and N.J. RUSSELL. *George Allen & Unwin:1984. Pp.162. Hbk ISBN 0-04-574021-6; pbk ISBN 0-04-574022-4. Hbk £19.50; pbk £7.95, $12.95.*

Membrane Dynamics and Transport of Normal and Tumor Cells. L. TRÓN *et al.* (eds). *Akadémiai Kiadó, Budapest:1984. Pp.387. ISBN 963-05-3889-X. £22.75.*

Membranes, Channels, and Noise. ROBERT S. EISENBERG, MARTIN FRANK and CHARLES F. STEVENS (eds). *Plenum:1984. Pp.288. ISBN 0-306-41806-1. $47.50.*

Methods in Enzymology. Vol. 105 Oxygen Radicals in Biological Systems. LESTER PACKER (ed.). *Academic:1984. Pp.600. ISBN 0-12-182005-X. £67.50, £52.*

Methods in Enzymology. Vol. 107 Posttranslational Modifications, part B. FINN WOLD and KIVIE MOLDAVE (eds). *Academic:1984. Pp.688. ISBN 0-12-182007-6. $69.50, £49.*

Methods in Marine Zooplankton Ecology. By MAKOTO OMORI and TSUTOMU IKEDA. *Wiley: 1984. Pp.332. ISBN 0-471-80107-0. £47.80.*

Methods in Microbiology, Vol. 14. T. BERGAN (ed.). *Academic:1984. Pp.228. ISBN 0-12-521514-2. $40, £27.*

Methods in Pharmacology. Vol. 5 Myocardial Biology. ARNOLD SCHWARTZ (ed.). *Plenum:1984. Pp.236. ISBN 0-306-41684-0. $42.50.*

Mode of Action of Antifungal Agents. A.P.J. TRINCI and J.F. RYLEY (eds). *Cambridge University Press: 1984. Pp.405. ISBN 0-521-26171-6. £42.50.*

The Molecular Biology of Adenoviruses 3. Current Topics in Microbiology and Immunology. WALTER DOERFLER (ed.). *Springer-Verlag:1984. Pp.110. ISBN 3-540-13138-8. DM 62, $24.30.*

The Molecular Virology and Epidemiology of Influenza. CHARLES H. STUART-HARRIS and C.W. POTTER (eds). *Academic:1984. Pp.305. ISBN 0-12-674740-7. $29.50, £19.50.*

Monoamine Innervation of Cerebral Cortex. Vol. 10 Neurology and Neurobiology. LAURENT DESCARRIES, TOMAS R. READER and HERBERT H. JASPER (eds). *Alan R. Liss:1984. Pp.361. ISBN 0-8451-2711-X. £52.*

Motility and Taxis in Prokaryotes. By A.N. GLAGOLEV. *Harwood:1984. Pp.279. ISBN 3-7186-0160-5. $126.*

Movement Disorders: Tremor. LESLIE J. FINDLEY and RUDY CAPILDEO (eds). *Macmillan, London:1984. Pp.493. ISBN 0-333-35040-5. £60.*

Nearctic Avian Migrants in the Neotropics. By JOHN H. RAPPOLE *et al. World Wildlife Fund:1983. Pp.646. Np.*

Neonatal Behavioral Assessment Scale. By T. BERRY BRAZELTON. *Blackwell Scientific:1984. Pp.125. ISBN 0-632-01263-3. £7.50.*

Neural Mechanisms of Startle Behavior. ROBERT C. EATON (ed.). *Plenum 1984. Pp.377. ISBN 0-306-41556-9. Np.*

The Neurobiology of Pain. ARUN V. HOLDEN and WILLIAM WINLOW (eds). *Manchester University Press:1984. Pp.414. ISBN 0-7190-1061-6. £35.*

Neurotransmitter Receptors: Mechanisms of Action and Regulation. SHOZO KITO *et al.* (eds). *Plenum:1984. Pp.297. ISBN 0-306-41729-4. Np.*

Neurotransmitters and the Cerebral Circulation. E.T. MACKENZIE, J. SEYLAZ and A. BÈS (eds). *Raven: 1984. Pp.270. ISBN 0-88167-010-3. $54.50.*

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PROTEIN KINASES (August 4 - 9)
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Opening Address: P. Greengard. **Evening Address:** I. Pastan. **Protein Kinase C.** J. Kuo, R. Bell, P. Blumberg, W. Anderson; **Calmodulin Protein Kinase.** R. Adelstein, A. Means, R. Tsien; **Phosphatases.** C. Klee, E. Fischer, D. Brautigan; **Substrates.** P. Blackshear, L. Williams, J. Garrison, J. Cooper; **Receptor Phosphorylation.** R. Lefkowitz, R. Klausner, P. Liebman; **Signal Generation.** E. Lapetina, J. Williamson, L. Cantley, M. Smigel; **Gene Regulation.** D. Granner, R. Maurer, M. Rosenfeld, M. Greenberg; **Receptor Kinases.** S. Aaronson, W. Pledger, R. Davis.

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Dynamic solidification of a binary melt

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The fundamental physics and fluid dynamics of a solidifying two-component system cooled from below is analysed quantitatively. Theoretical results for the rate of growth of the solid are in good agreement with experimental measurements on crystallizing aqueous solutions. The results are applicable to the solidification of a wide variety of binary alloys.

THE cooling and resultant solidification of a melt is important in many different fields, including crystal growth[1,2], electrical engineering[3,4], geology[5,6], geophysics[7,8], metallurgy[9,10] and oceanography[11,12]. The investigation of the fluid dynamic processes which often dominate solidification has been advanced by the suggestion[13-15] that the mechanisms may be elucidated by laboratory experiments on the freezing of aqueous solutions. In some cases[14,15] the studies were aided greatly by the concurrent analysis of a predictive mathematical model. Here, we use this powerful approach and describe some simple experimental and associated theoretical models for the cooling and crystallizing at a horizontal boundary of an initially homogenous fluid. The aim is to highlight the fundamental phenomena and to minimize consideration of inessential details by studying a simple and straightforward situation. We use aqueous solutions because they are easy to handle in the laboratory, but the concepts developed are applicable directly to the solidification of a wide variety of binary alloys.

First, we discuss briefly the phase diagram of the solid/melt mixture and then concentrate on effects associated with cooling and solidifying a melt from below. We determine theoretically the rate of solidification if the solid/melt interface is perfectly flat and horizontal, and also evaluate the criterion for which the interface becomes unstable. In a series of laboratory experiments, we have grown ice with unstable interfaces from different aqueous solutions and we have developed a simple theoretical model whose predictions are in good agreement with the observations. Our main quantitative conclusions are theoretical and experimental relationships for the rate of growth of the crystal block, the total volume of solid produced and an expression for the volume fraction of the solid product.

Phase diagram

The chemical composition of the phase obtained by the solidification of a melt with two chemical components is determined at thermodynamic equilibrium by the phase diagram (Fig. 1). This diagram represents the chemical compositions of melt and solid in equilibrium with each other by the liquidus and solidus respectively. At a temperature and composition represented by a point above the liquidus, the system is liquid. Between the liquidus and the eutectic line, the solidus defines the composition of the material solidifying from the melt, and in general specifies solid phases whose composition differs from both the melt and the pure components of the system[6,9]. For most aqueous solutions and many binary alloys, however, the phase diagram has the special form illustrated in Fig. 1*a*, where solidification from a melt (solution) with sub-eutectic composition yields a solid phase consisting of a pure component (ice). It is usually the case that density in the melt is a much stronger function of composition than of temperature (see Fig. 1), which plays an important role in the fluid dynamics of solidification.

The solidification of a melt of eutectic composition, yielding solid of the same bulk composition, is equivalent to the solidification of a pure material. Otherwise, study of the process of solidification requires that melts with compositions greater and less than that of the eutectic must be dealt with separately, while the direction of cooling (from above or below) also requires distinctive treatment. Table 1 shows the 3×2 matrix by which the problems of solidification may be classified. What follows concentrates mainly on cooling from below of a melt whose composition is below the eutectic value.

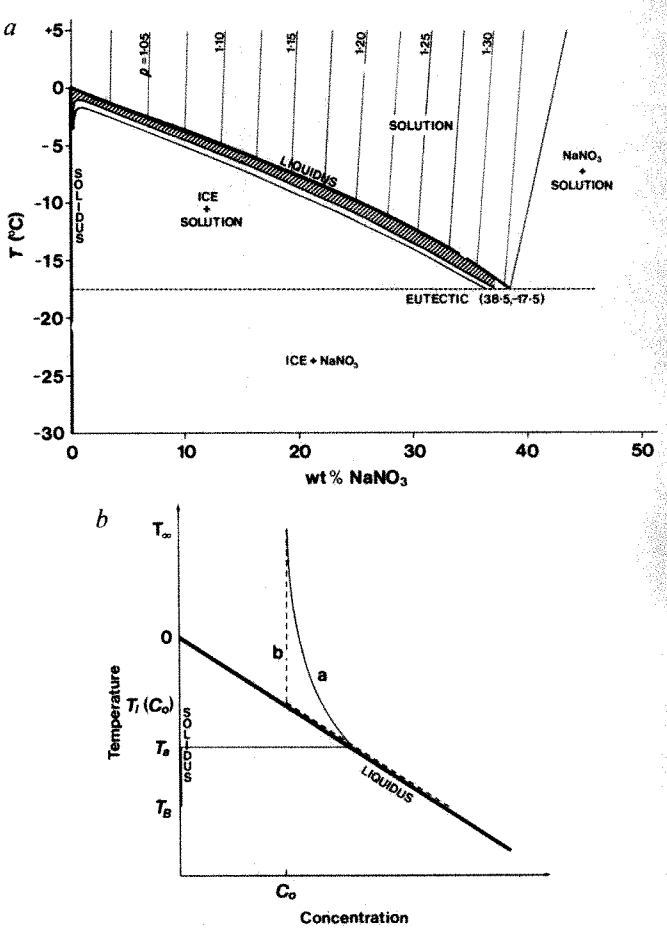


Fig. 1 *a*, Phase diagram for an aqueous solution of $NaNO_3$ (refs 20, 24) with axes of temperature and concentration. The same diagram acts as a schematic for a general binary alloy with components A and B. The figure also presents the lines of constant density with the density values attached. Hatched region, morphologically stable region for $T_\infty = 15$ °C. The curve just beneath the hatched region indicates the curve of marginal stability for $T_\infty = 30$ °C. In determining the curves the following values of the constant physical parameters have been used: $C_{p_m} = 1.0$ cal g^{-1} °C^{-1}, $C_{p_s} = 0.48$ cal g^{-1} °C^{-1}, $D = 10^{-5}$ cm^2 s^{-1}, $L = 80$ cal g^{-1}, $\kappa_m = 0.0013$ cm^2 s^{-1}, $\kappa_s = 0.012$ cm^2 s^{-1}, $\rho_s = 0.916$ g cm^{-3}. Further, the values of Γ and ρ_m were obtained as a function of C_0 from ref. 20. *b*, Phase diagram used here, with temperature and concentration axes chosen so that a solid of zero concentration forms at zero temperature. Heavy solid line, straight-line approximation to the liquidius. Thin curve a, temperature against concentration profile in the morphologically stable regime. Dashed curve b, approximate profile used in our model of the morphologically unstable regime.

Table 1 The six different cases that arise when an initially homogeneous solution is cooled at a horizontal boundary

	Cool from Above	Cool from Below	
$C > C_E$	13	25	Light fluid released
$C = C_E$	13	14	Uniform composition throughout
$C < C_E$	22	here	Heavy fluid released
	Thermally unstable	Thermally stable	

Numbers refer to the reference in which each case is discussed. See text for explanation of symbols.

Eutectic growth

The solidification of a melt of eutectic composition involves only variations in temperature, because the composition in the melt and solid is constrained to remain at the eutectic value. This situation is equivalent to one described by Carslaw and Jaeger[16], who present the analytical solution for a column of melt of infinite height whose initial temperature is T_∞ and which is cooled at a lower boundary whose temperature is T_B. We conducted two experiments using eutectic compositions of aqueous Na_2CO_3 (eutectic composition $C_E = 5.7$ wt%, eutectic temperature $T_E = -2.1$ °C) in an insulated Perspex tank 20 cm × 20 cm × 45 cm high. Our observations of the height of the compact eutectic solid as a function of time in one of our experiments are plotted in Fig. 2. They are in good agreement with the analytical solution, which attests to the reliability of our experimental methods.

Sub-eutectic growth

We have extended the study of the solidification of a eutectic melt to the case where the composition is not that of the eutectic but in which the initial uniform concentration of solute C_0 is less than C_E. Solid of composition $C = 0$ grows on the cooled boundary and, to begin with, we assume that the solid/melt interface remains flat and parallel to the boundary. The fluid released by the crystallization is relatively heavy and thus remains just above the interface, so that the concentration gradient which develops is stable. Thus, no physical motion takes place and the transport of heat and solute is by molecular diffusion alone. The governing equations are then those for heat transport (in one dimension, taken as z measured upwards from the cooled boundary) above and below the interface and for solute diffusion in the melt. The equations for the temperature $T(z, t)$ and the composition $C(z, t)$ are

$$\rho_s C_{p_s} \frac{\partial T}{\partial t} = k_s \frac{\partial^2 T}{\partial z^2} \quad \text{(for } z < h(t), \text{ the position of the moving boundary)} \tag{1}$$

$$\left.\begin{aligned} \rho_m C_{p_m} \frac{\partial T}{\partial t} &= k_m \frac{\partial^2 T}{\partial z^2} \\ \frac{\partial C}{\partial t} &= DL \frac{\partial^2 C}{\partial z^2} \end{aligned}\right\} (z > h(t); \text{ that is, for } z \text{ in the melt}) \tag{2, 3}$$

$$T = T_B \qquad (z = 0) \tag{4}$$

$$T \to T_\infty, \quad C \to C_0 \qquad (z \to \infty \text{ or } t \to 0) \tag{5}$$

The physical parameters are the solute diffusivity D, the thermal conductivity k, the density ρ and the specific heat C_p, all of which are considered to be constant. Subscripts s and m refer to properties of the solid and the melt, respectively. The difference in heat flux across the interface $z = h(t)$ results from the latent heat released on solidification. This can be expressed by

$$\rho_s L\dot{h} = -k_m \frac{\partial T}{\partial z}(h+, t) + k_s \frac{\partial T}{\partial z}(h-, t) \tag{6}$$

where a dot denotes a time derivative and L is the latent heat of fusion. Conservation of solute requires

$$C(h+, t)\dot{h} = -D \frac{\partial C}{\partial z}(h+, t) \tag{7}$$

Further, with the assumption that the solidification kinetics can be described by equilibrium thermodynamics, we require that the temperature and composition at the interface lie on the liquidus, which we approximate by the linear relationship

$$T = T_L(C) = -\Gamma C \tag{8}$$

This tacitly assumes, merely for convenience, that the liquidus temperature is 0 at zero composition, as it is for all aqueous solutions.

The system of equations and boundary conditions represented by equations (1)–(8) admits a similarity solution, with variable

$$\eta = \tfrac{1}{2}(Dt)^{-1/2} z \tag{9}$$

in which the interface has position

$$h = 2\lambda(Dt)^{1/2} \equiv \gamma_C^{t^{1/2}} \tag{10, 10'}$$

This similarity form of solution can be shown to be the only solution of the equations. The temperature and concentration fields, determined from equations (1)–(5), can then be expressed in terms of error functions[17] as

$$T(z, t) = T_B + (T_a - T_B)\,\mathrm{erf}\,(\varepsilon_S \eta)/\mathrm{erf}\,(\varepsilon_s \lambda) \qquad (z < h) \tag{11}$$

$$\left.\begin{aligned} T(z, t) &= T_\infty + (T_a - T_\infty)\frac{\mathrm{erfc}\,(\varepsilon_m \eta)}{\mathrm{erfc}\,(\varepsilon_m \lambda)} \\ C(z, t) &= C_0 + (C_a - C_0)\frac{\mathrm{erfc}\,\eta}{\mathrm{erfc}\,\lambda} \end{aligned}\right\} (z > h) \tag{12, 13}$$

where T_a and C_a are the temperature and concentration of the melt at the interface. There are two parameters $\varepsilon_{s,m} = (D/\kappa_{s,m})^{1/2}$, where $\kappa = k/\rho C_p$ is the thermal diffusivity. The interface conditions in equations (6)–(8) are now used to determine the eigenvalue λ from

$$\Gamma C_0 \left[\frac{F(\lambda)}{1 - F(\lambda)}\right]\left[\frac{\rho_m C_{p_m}}{F(\varepsilon_m \lambda)} + \frac{\rho_s C_{p_s}}{G(\varepsilon_s \lambda)}\right] = \frac{\rho_s C_{p_s} T_1}{G(\varepsilon_s \lambda)} - \frac{\rho_m C_{p_m} T_0}{F(\varepsilon_m \lambda)} - \rho_s L \tag{14}$$

where

$$F(x) = \pi^{1/2} x e^{x^2}\,\mathrm{erfc}\,x, \qquad G(x) = \pi^{1/2} x e^{x^2}\,\mathrm{erf}\,x$$

$$T_1 = T_L(C_0) - T_B, \qquad T_0 = T_\infty - T_L(C_0)$$

The solute diffusivity D is typically much smaller than the thermal diffusivity κ. In this case, $\varepsilon \ll 1$, and in the limit $\varepsilon \to 0$ equation (14) reduces to the simpler expression

$$F(\lambda) \approx [1 + (\Gamma C_0/T_1)]^{-1} = (C_a - C_0)/C_0 \tag{15, 15'}$$

Because F increases monotonically from 0 to 1, equation (15) has a unique solution of λ of order unity. This confirms the scaling used in equation (10) and indicates that the growth of the flat interface is controlled by solute diffusion. Note, however, that when C_0 is small (of the order of $\varepsilon^2 = D/\kappa$, with κ_s/κ_m of the order of unity) the growth is no longer controlled by solute diffusion but rather by thermal diffusion. This is indicated by the fact that in this event $\lambda = O(\varepsilon^{-1})$ and so $h = O(\kappa t)^{1/2}$. It would be interesting to carry out an experimental verification of the validity of equation (14).

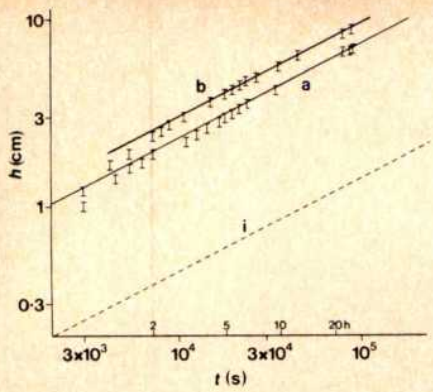


Fig. 2 Experimental and theoretical data on the height of the block as a function of time. Line a, an experiment using a Na_2CO_3 solution at eutectic composition with $T_B = -5.7$ °C, $T_\infty = 17.3$ °C. Line b is the representative experiment R with parameters in the morphologically unstable regime. Line b is the prediction of the theoretical model whereas line i is the predicted height of a flat interface at the same conditions.

Morphological instability

If the undercooling, $T_L - T_B$, is too large, solidification proceeds too quickly and the solidification interface is no longer planar. It grows in a spatially irregular manner, which will be described photographically in more detail below. The initial break-up of the planar interface is often called morphological instability. A useful physical description of the instability is discussed at length by Langer[18]. Detailed calculations[19] indicate that, on the assumption that surface tension effects are negligible, instability occurs when

$$k_s \frac{\partial T}{\partial z}(h-, t) + k_m \frac{\partial T}{\partial z}(h+, t) = -(k_s + k_m)\Gamma \frac{\partial C}{\partial z}(h+, t) \quad (16)$$

If thermal conductivity in the solid is neglected ($k_s = 0$), equation (16) becomes equivalent to the statement that instability arises whenever the predicted values of temperature and composition on the melt side of the interface lie beneath the liquidus (where the system should be partially solid). The diffusion of heat in the solid away from the interface somewhat alters the onset of instability. Surface tension does not alter equation (16) significantly in most practical situations, so we may use it as a good approximate criterion and express it in terms of our similarity solution as

$$T_0 < \Gamma C_0 \left[\frac{F(\lambda)}{1 - F(\lambda)}\right]\left[\frac{k_m + k_s}{2k_m}\frac{F(\varepsilon_m \lambda)}{\varepsilon_m^2 F(\lambda)} - 1\right] - \frac{1}{2}\frac{\rho_s}{\rho_m}\frac{L}{C_{p_m}} F(\varepsilon_m \lambda) \quad (17)$$

For given values of T_∞ and C_0 the value of T_B at which supercooling first occurs is found by solving equation (17) simultaneously with equation (14). The critical value of T_B is plotted as a function of C_0 for two values of T_∞ in Fig. 1*a*.

Experiments

We performed 12 experiments with various solutes at different values of T_B and C_0 in the tank described above. Temperatures were monitored continuously with thermistors, some of which were held fixed, and the ice block allowed to freeze around them. Occasional measurements of concentration were made by withdrawing small samples and analysing them with a hand-held refractometer. All the experiments were in the morphologically unstable regime.

The results of a representative experiment, denoted by R ($NaNO_3$; $T_B = -16.5$ °C, $T_\infty = 14.7$ °C and $C_0 = 14$ wt%), are shown in Figs 2–4. Figure 3 shows three views of the solid ice block after it was removed from the tank. The humpy form of

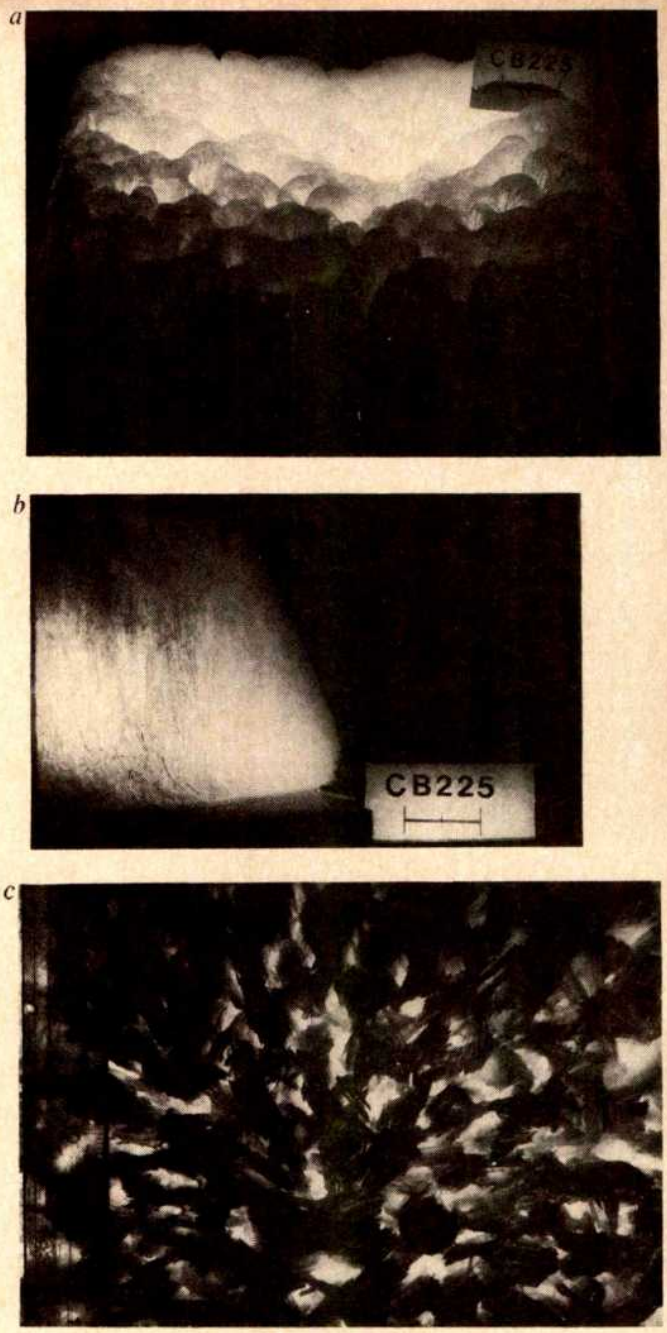


Fig. 3 *a*, An oblique view of the top of the ice block in experiment R showing the humpiness of the surface on a scale of centimetres and the finer scale 'facets' on a scale <1 mm. Horizontal scale, 2 cm long. *b*, Vertical view of the same experiment showing the lines of solid ice and the compositionally-enriched fluid. *c*, Close-up view looking vertically downwards onto the top of the block in the same experiment. Note the very fine-scale irregularities.

the surface (Fig. 3*a*) and the small-scale facets (Fig. 3*c*) depict cearly that the system is in the morphologically unstable regime. Figure 2 presents the (maximum) height of the block as a function of time and compares this with the result obtained by solving equations (10) and (14). The difference between the two indicates that the contortions of a morphologically unstable surface allows it to grow much more rapidly than if it were to remain stable. Figure 4 presents experimental and theoretical data on temperature and concentration at various times. There is considerble difference between the experimental results and those given by equations (11)–(14). The generality of this disagreement is confirmed in Fig. 5 which compares the experimental values of γ with those given by equations (11)–(14) for varying initial C_0 at fixed T_B and varying T_i at fixed C_0.

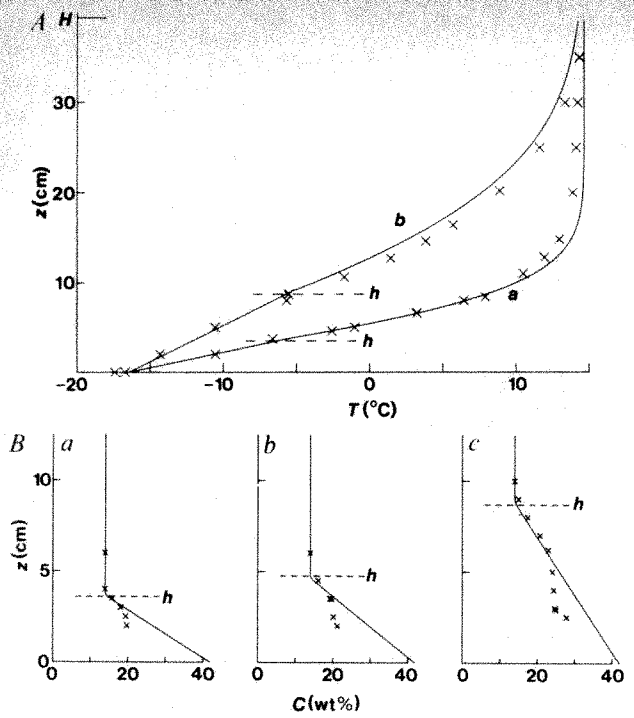


Fig. 4 *A*, Temperature profiles as a function of depth for experiment R at *a*, 3 h 40 min and *b*, 25 h 35 min. Crosses, data points; dashed lines, observed heights, h, of the ice block. Solid curves, temperatures predicted by the model of the mush. *B*, Composition profiles as a function of depth for experiment R at *a*, 3 h 20 min; *b*, 7 h 0 min; *c*, 25 h 20 min. Crosses, $NaNO_3$ concentration in fluid withdrawn by means of inserting a thin sampling tube; dashed lines, observed heights, h, of the ice block; solid curves, concentrations predicted by the model of the mush.

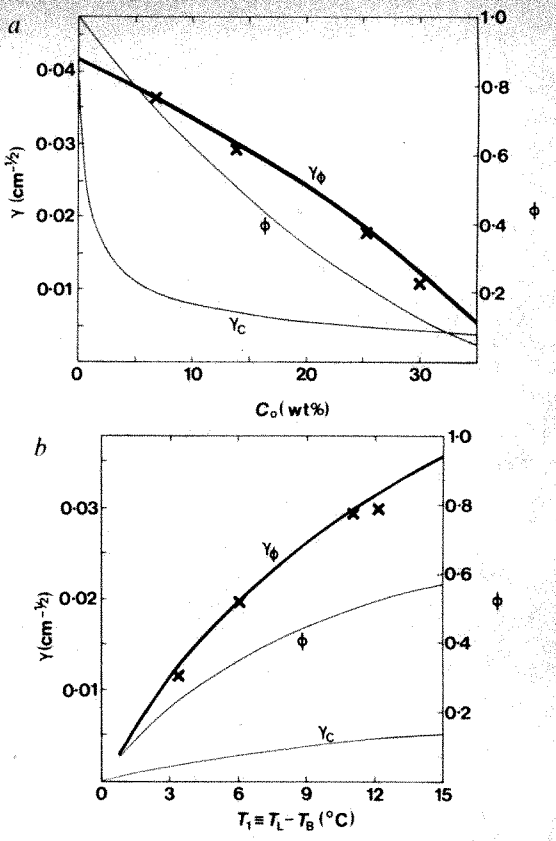


Fig. 5 *a*, Growth rate coefficient (γ) and solid fraction (ϕ) as functions of concentration for $T_B = -17\,^\circ C$ and $T_\infty = 15\,^\circ C$. The crosses are experimental values of λ. The heavy curve is the theoretical prediction obtained by solving equations (22) and (23). The light curve γ_C is the result of the model which assumes a flat interface and results in equation (14). *b*, γ and ϕ as a function of the undercooling $T_i = T_L - T_B$ for $C_0 = 14$ wt% and $T_\infty = 15\,^\circ C$.

Theoretical model

The experimental observations described here suggest that a mixed phase of solid and melt, sometimes called a mush phase, is formed when the flat interface is morphologically unstable. Equations describing the evolution of a mush have been developed previously[21,22] and similarity solutions have been found[21]. These equations relate bulk properties of the mush, averaged over a length scale larger than the typical crystal spacing. Thus, when a melt is cooled from below and crystallizes in such a way that relatively heavy fluid is released, the averaged equations predict convective stability of the interstitial fluid. The large pore size in the ice-blocks, however, suggested that compositional convection resulting in solute redistribution had occurred on a sub-pore scale. This would render the previous models inapplicable to the present situation. However, a simple predictive model of the growth of the mush (when $T_B > T_E$) can be developed, based on the controlling nature of thermal diffusion and the appropriate use of bulk conservation relationships.

We assume, for simplicity, that the densities of the solid and melt are the same. The mush extends from the cooled boundary to some position $z = h(t)$ and we assume the solid fraction, ϕ, within the mush to be constant. In reality, ϕ varies with height, but our model conserves mass only on a global scale and the constancy of ϕ is a necessary and consistent approximation. The solute concentration has uniform value C_0 throughout the melt in $z > h$ and obeys the liquidus relation described by equation (8) in the interstices of the mush. Diffusion governs the heat transfer in both the mush and the melt. Thus the thermal field is given by equations (11) and (12) with k_s and C_{p_s} replaced by the approximate expressions, whose validity are discussed by Batchelor[23]

$$\bar{k} = \phi k_s + (1-\phi)k_m \tag{18}$$

and

$$\bar{C}_p = \phi C_{p_s} + (1-\phi)C_{p_m} \tag{19}$$

and T_a replaced by $T_L(C_0)$. Global conservation of solute requires that

$$(1-\phi)\int_0^{h(t)} C\,dz = -\Gamma^{-1}(1-\phi)\int_0^{h(t)} T\,dz = h(t)C_0 \tag{20}$$

while conservation of heat at the mush/melt interface is expressed by

$$\rho L\phi\dot{h} = -k_m\frac{\partial T}{\partial z}(h+,t) + \bar{k}\frac{\partial T}{\partial z}(h-,t) \tag{21}$$

Expressions (11) and (12) are used in equations (20) and (21) to obtain

$$\phi = \left[1 + \frac{\Gamma C_0}{T_i}H(\bar{\delta}\mu)\right]^{-1} \tag{22}$$

$$L\phi = \frac{-C_{p_m}T_0}{F(\mu)} + \frac{\bar{C}_p T_i}{G(\bar{\delta}\mu)} \tag{23}$$

where

$$H(x) = [\exp(x^2) - 1]^{-1}G(x)$$

$$\bar{\delta} = (\kappa_m \rho \bar{C}_p/\bar{k})^{1/2}$$

and

$$\mu = \varepsilon_m \lambda$$

In terms of the new variable μ, the height of the block is given by

$$h = 2\mu(\kappa_m t)^{1/2} = \gamma_\phi t^{1/2} \tag{24, 24'}$$

which confirms that the growth of the block is governed by thermal diffusion. Note, alternatively, that solute diffusion is ignored in the model and correspondingly D does not appear in equations (22), (23) or (24).

Equations (22) and (23) can be solved simultaneously for ϕ and μ. The growth rate coefficient γ_ϕ is plotted as a function of C_0 for fixed T_B in Fig. 5*a* and as a function of T_B for fixed C_0 in Fig. 5*b*. The predicted growth rate is compared with the experimental results in Figs 2 and 5 and the agreement is good.

The measured temperature profiles at two different times during experiment R compare well with the predicted temperatures (Fig. 4*A*) and the measured concentration profiles agree reasonably well (Fig. 4*B*). The disagreement between the experimental data and the theoretical model at points deep in the block may result from the difficulty of inserting a sampling tube reliably to that depth and withdrawing fluid for the concentration measurement.

The validity of the theoretical relationships was tested further by conducting some experiments with different aqueous solutions. The experimental results obtained from using NaCl and NH_4Cl, which have different liquidus relationships and diffusion coefficients, again showed good agreement with the results of the theoretical model.

In conclusion, the experiments and related theory indicate that accurate predictions can be obtained from the use of simple conservation relationships when a melt whose composition is less than the eutectic is cooled from below. Variations of this same approach allow all six different cases, as mapped out in Table 1, to be solved. We plan to publish further details elsewhere.

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1 Brice, J C *The Growth of Crystals from Liquid* (North-Holland, Amsterdam, 1973)
2 Bardsley, W, Hurle, D T J & Mullin, T B *Crystal Growth a Tutorial Approach* (North-Holland, Amsterdam, 1979)
3 Laudise, R A *The Growth of Single Crystals* (Prentice-Hall, New York, 1972)
4 Rosenberger, F *Fundamentals of Crystal Growth* Vol 1 (Springer, Berlin, 1979)
5 Huppert, H E & Sparks, R S J *A Rev Earth planet Sci* **12**, 11-37 (1984)
6 Ehlers, E G *The Interpretation of Geological Phase Diagrams* (Freeman, San Francisco, 1972)
7 Gubbins, D, Masters, T G & Jacobs, J A *Geophys J R. astr Soc* **59**, 57-99 (1979)
8 Loper, D E & Roberts, P H in *Stellar and Planetary Magnetism* (ed Soward, A M) 297-327 (Gordon & Breach, London, 1983)
9 Flemings, M C *Solidification Processing* (McGraw-Hill, New York, 1974)
10 Chalmers, B *Principles of Solidification* (Wiley, New York, 1964)
11 Hobbs, P V Ice Physics (Clarendon, Oxford, 1974)
12 Fletcher, N H *The Chemical Physics of Ice* (Cambridge University Press, 1970)
13 Chen, C F & Turner, J S *J. geophys Res* **85**, 2573-2593 (1980)
14. Huppert, H E & Turner, J S *Earth planet Sci Lett* **54**, 144-152 (1981)
15 Turner, J S, Huppert, H E & Sparks, R S J *J Petrol* (in the press)
16 Carslaw, H S & Jaeger, J. C *Conduction of Heat in Solids* (Oxford University Press, 1959)
17 Abramowitz, M & Stegun, I A *Handbook of Mathematical Functions* (US Government Printing Office, Washington, 1964)
18 Langer, J S *Rev mod Phys* **52**, 1-28 (1980)
19 Sekerka, R F *Crystal growth an Introduction* (ed Hartman, P) 403-442 (North-Holland, Amsterdam, 1973)
20 Weast, R C (ed) *Handbook of Chemistry and Physics* (CRC, Cleveland, 1971)
21 Worster, M G thesis, Univ Cambridge (1983)
22 Hills, R N, Loper, D. E & Roberts, P H *Q. Jl Mech appl Math* **36**, 505-539 (1983)
23 Batchelor, G K *A. Rev Fluid Mech* **6**, 227-255 (1974)
24 Washburn, E W (ed) *International Critical Tables* (McGraw-Hill, New York, 1929)
25 Kerr, R C thesis, Univ Cambridge (1984)
26 Weeks, W F & Ackley, S F. *Cold Regions Res Engng Lab Mon* 82-1 (1982)

Cordilleria, a newly defined Canadian microcontinent

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Palaeomagnetic data and geological field evidence indicate that most of the western Canadian cordillera lay 1,500 km south of its present position in the early Cretaceous. We suggest that this region comprised a microcontinent, termed Cordilleria, which moved on the Kula plate and collided with the craton ~100 Myr ago to form first the Mackenzie Mountains and then the Rockies.

MODELS for the evolution of the North American cordillera have proliferated since the first attempt[1] at a plate-tectonic synthesis for western North America, based on the classical fivefold division of the cordillera in British Columbia. Earlier models concentrated on the history of palaeosubduction zones; later a long list of dextral transcurrent faults of regional significance were added[2,3] and integration of the United States and Canadian portions of the jigsaw attempted[3,4]. More than 50 microplates and terranes are now recognized[5]. Palaeontological[5-10] and palaeomagnetic (refs 11-16 and E. Irving, G. J. Woodsworth and P. J. Wynne, manuscript in preparation) data clearly show the necessity for major Mesozoic transcurrent movement.

The earlier palaeomagnetic data[11-13] indicated that elements of superterrane Terrane II (ref. 17; Fig. 1) were far travelled. More recent data (refs 14, 15 and E. Irving *et al.*, in preparation) show that Quesnellia and Stikinia, elements of Terrane I (ref. 17), are also far travelled, consistent with the palaeontological evidence[7,8]. The most recent palaeomagnetic evidence (refs 15, 16 and E. Irving *et al.*, in preparation) suggests that both Terranes I and II have been displaced northwards by 14-20° of latitude and rotated clockwise through 45-50° with respect to the North American craton since the early to mid-Cretaceous. These data, from two laboratories and a wide range of Triassic, Jurassic and Cretaceous rocks, show that the southern half of British Columbia was at the present-day relative latitude of California in the early Jurassic, and was probably still there in the early to mid-Cretaceous. The movement to be accommodated between Terranes I and II and the North American craton is ~1,500 km since the mid-Cretaceous. The locus of a possible innermost (eastern) transcurrent fault on which to take up this movement has remained a problem[3,17]. Recent palaeomagnetic evidence is difficult to reconcile with the relatively small geologically observable transcurrent movements on any of the major cordilleran faults.

Transcurrent faults

The major transcurrent faults of the Canadian cordillera are shown in Fig. 2. The Denali, Queen Charlotte and Yalakom-Pasayten fault systems are too far west to accommodate movement of any elements of Terrane I. The Finlay fault system (Kutcho-Kechika-Finlay-Pinchi faults) has a total offset of ~300 km[18] and no true southern extension. The Fraser fault system can accommodate only ~150 km of early Tertiary move-

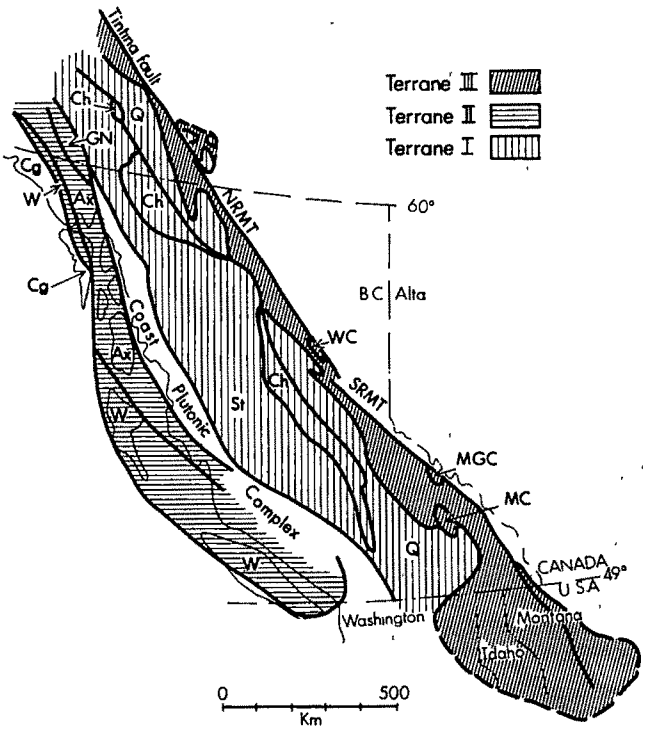


Fig. 1 Part of the Canadian cordillera, showing Terranes I and II of Monger *et al.*[17] and Terrane III proposed here. Ax, Alexander terrane; Cg, Chugach terrane; Ch, Cache Creek terrane; GN, Gravina–Nutzotin terrane, Q, Quesnellia; St, Stikinia; W, Wrangellia. M, Monashee complex; MGC, Malton gneiss complex; WC, Wolverine complex; NRMT, Northern Rocky Mountain Trench; SRMT, Southern Rocky Mountain Trench.

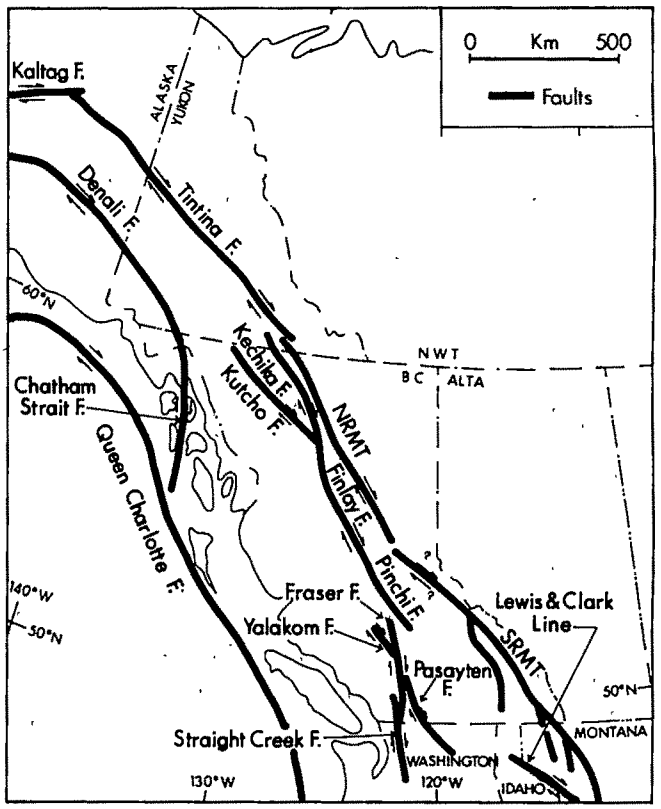


Fig. 2 Main transcurrent faults of the Canadian cordillera. None shows sufficient movement to account for all the palaeomagnetically deduced motion.

ment, which is a very small fraction of the total northward movement required of Stikinia (ref. 3 and J. W. H. Monger, personal communication). The Tintina fault and the North Rocky Mountain Trench (NRMT) fault together form a system ~1,500km long. 450 km of late Cretaceous dextral strike slip movement has been documented on the Tintina fault[19] and there may have been as much as 1,000 km of post-Palaeozoic movement on the Tintina/NRMT system (H. Gabrielse, personal communication). Thus, at least some of the northward movement of Terranes I and II may have been taken up on these faults.

Southern Rocky Mountain Trench

The Southern Rocky Mountain Trench (SRMT) is almost colinear with the Tintina and NRMT faults, although there is a discontinuity at 54° N. Together, these three faults form one of the major linear features of the Earth's crust, with a total length of ~2,500 km. The exceptional length and linear character of the SRMT have attracted many suggestions of Mesozoic or Palaeozoic transcurrent movement along it[20]. The large erosional feature (trench) suggests that it is the locus of a major fault, but its surface expression at the present-day stratigraphic level seems to result from thrust and dip-slip faulting[21]. First motion studies on a ~14-km deep, 4.8-mag earthquake just east of the SRMT near Valemount in British Columbia in 1978, however, showed dextral transcurrent as well as thrusting movement[22]. Faults frequently contain more than one type of movement[23].

Malton gneiss complex

Most stratigraphic correlations made across the SRMT are carried on thrust slices, but one major anchor seemed to be the Malton gneiss complex, which sits astride the trench at latitude 52°30'N, and has been interpreted as a single gneissic body of Proterozoic age[24]. As such, it prohibited any large-scale post-Proterozoic movement on the SRMT.

Our investigations[25–31], which included major and minor element analysis of 250 gneiss samples, have shown that the Malton gneiss complex consists of several distinct fault-bounded blocks of amphibolite facies gneiss, each of different age, geological history and petrogenesis (Fig. 3). This is supported by other authors, who describe all contacts between the gneissic basement and the Proterozoic sedimentary cover as faults, thrusts or decollements[30–32]. In particular, the Malton Block to the west of the SRMT differs from the three gneissic blocks to the east of the SRMT in age, lithology, geochemistry and isotope composition[25–29].

These fundamental differences between the gneisses east and west of the SRMT mean that the Malton gneiss complex is almost certainly more than one gneissic complex and can no longer be regarded as a constraint to transcurrent movement along the SRMT, nor as a tie, linking stratigraphy on one side of the SRMT to that on the other side. The gneisses do not correlate across the trench. For this reason, we have suggested that the term 'Malton gneiss complex' to describe the blocks east of SRMT should no longer be used[29].

Researchers who have studied the SRMT elsewhere have failed to uncover such significant differences across it, so we suggest that the Malton Block may be basement exposed in a window through the overlying late Cretaceous to Tertiary thrust sheets. Proterozoic metasediments of the Horsethief Creek Group are thrust over the Malton gneiss and across the SRMT[33]. A major decollement between the Proterozoic metasediments of the Kaza Group and the underlying Malton gneiss exposed on top of the Malton Block near the head of Robina Creek (Fig. 3) may be the basal decollement of the Rockies. The gneissic blocks east of the SRMT seem to be thrust slices, but have no obvious correlatives to the west. The reason for basement exposure at this location may be a domal upwarp at the intersection of two major structural antiforms. These are the NNW–SSE trending line of domal metamorphic core complexes[34] and the WSW–ENE trending Jasper culmination[35] that brings Precambrian rocks to the surface across the entire width of the main ranges at this latitude. The metamorphic grade of the Malton gneiss complex indicates that the surface presently

exposed has moved upwards by at least 15 km since major metamorphism.

Geophysical evidence

As a result of our observations, particularly the 'window into basement' concept, and the data demonstrating the differences across the SRMT at 52°30′N, we have reviewed the geophysical evidence for the deep structure of the crust and lithosphere on either side of the SRMT. North of ~52°N, every major geophysical parameter undergoes a significant, usually abrupt, change from one side of the SRMT to the other. South of ~52°N, these geophysical changes seem to follow a north–south line up to 150 km west of the present surface expression of the SRMT and close to the line of Kootenay Lake. The structure of the lithosphere is fundamentally different east and west of this SRMT–Kootenay Lake line, shown by seismic, gravity, magnetic, electrical conductivity and heat-flow data. The seismic activity along the SRMT is moderate, with at least one earthquake of magnitude ≥3 every 2 yr, on an average based on the past 20 yr. In 1918, there was a 6.0-mag earthquake south of the Hugh Allan Creek block, and in 1978 a 4.8-magnitude earthquake in the Bulldog Creek area[36].

The thickness of both the lithosphere and the crust change markedly across this SRMT–Kootenay Lake line, both being much thinner to the west. The crust has an average thickness of 50 km east of the SRMT[37] and 35 km west of the SRMT[38–40]. The lithosphere west of the SRMT is also 35 km thick, its base apparently coincident with the base of the crust[39,41]. The wider spacing of the magnetic anomalies east of this line[42] is consistent with the lithosphere there being thicker than to the west. The abrupt change in the trend of the magnetic anomalies as this line is crossed suggests strongly a fundamental lithospheric break[42]. Details of the magnetic anomalies such as a broadening of the eastern anomalies at the SRMT suggest a cratonic or lithospheric plate margin. The magnetically quiet zones to the west of the SRMT–Kootenay Lake line and east of the Queen Charlotte fault are also typical of plate boundaries; the magnetic zone boundaries[42] suggest that there exists a single separate plate between the Pacific plate and the Rocky Mountain Trench. This plate, we suggest, is a remnant of the Kula plate. Further evidence for the eastern boundary of this plate comes from electrical conductivity[43] and heat flow[44] measurements, both of which decrease sharply as the SRMT–Kootenay Lake line is crossed from west to east. Recently, a narrow region of exceptionally high electrical conductivity has been measured to the east of the SRMT under the main ranges of the Rocky Mountains near Valemount (D. I. Gough, in preparation). This is also a broad area of extremely high negative Bouguer anomaly (210 mGl) which is centred over the southern Canadian Rocky Mountains and decreases north-west and south-east around latitudes 54°N and 49°N, as well as decreasing to the north-east and south-west along the margins of the Rocky Mountain ranges[45].

The detailed structure of the lithosphere at lower crustal and mantle depths changes abruptly at the SRMT, shown by seismic and gravity measurements. A section across the SRMT at latitude 50°30′N, accommodating both kinds of data, shows the SRMT as a vertical fault at depth, but covered at upper crustal levels by continuous very low-density (2.76) material[46]. It also shows a high-density (3.03) lower crustal zone to the east of the SRMT and disappearing at the trench, and a high-density (3.43) upper mantle zone, present to the east, terminating abruptly at the trench. Both are replaced to the west by 3.36 density upper mantle material. This model suggests that the SRMT is a vertical fault at depth, separating lithospheres of contrasting structures. The continuous low-density material extending laterally across the vertical fault at upper crustal levels we interpret as the Rocky Mountain thrust sheets. We suggest that the lithosphere structure here may be similar to that illustrated for the closure of the Iapetus Ocean[47]. The continental crust of England is there shown being overriden by thrust sheets from Scotland, the plane of

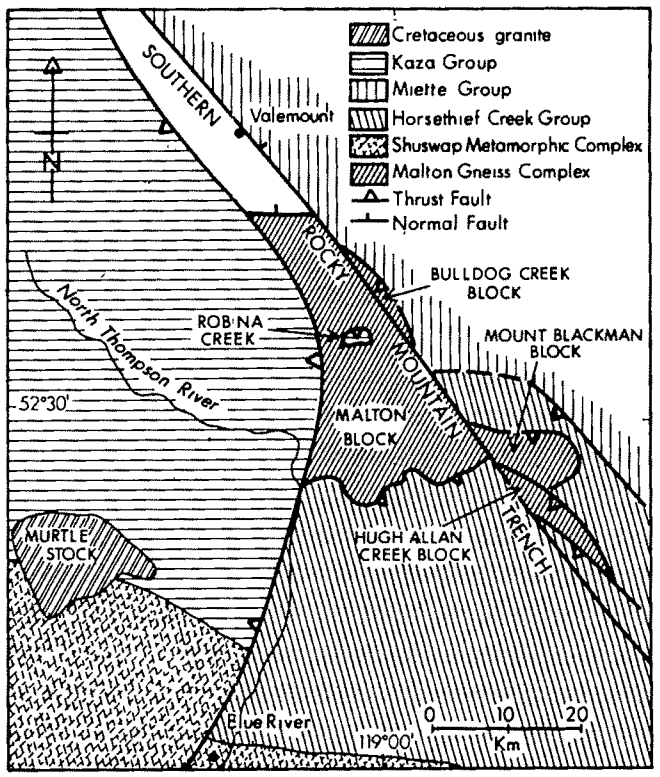


Fig. 3 The Malton gneiss complex, showing the separate blocks lying east and west of the SRMT and the overlying thrust sheets of the Proterozoic Kaza and Horsethief Creek groups.

suture dipping at a shallow angle to the north with the geophysical boundary ~100 km north of the suture in the cover rocks.

Terrane III

The Canadian cordillera has been described in terms of two superterranes, Terrane I and Terrane II, the former having amalgamated by latest Triassic–earliest Jurassic and the latter by late Jurassic[17]. The Omineca crystalline belt and the coast plutonic complex are described as two metamorphic and igneous welts that developed as a result of the collisions of these superterranes. The coast plutonic complex formed as a result of the accretion of Terrane II to Terrane I during the Cretaceous. The Omineca crystalline belt resulted from the mid-Jurassic collision of Terrane I with continental crustal rocks to the east[17].

The rocks with which Terrane I collided during the mid-Jurassic include the north-tapering wedge of Proterozoic and Phanerozoic metasediments of the Purcell, Windermere, Hamill, Lardeau and Broadview groups, the ancient gneissic rocks of the Monashee complex (basement to the Monashee decollement[48]) and possible also the Malton and Wolverine blocks. Further north, the Teslin fault is the suture zone of the mid-Jurassic collision[49], welding Terrane I to the Anvil range and Klondike schist groups.

There is no evidence of large-scale post-Jurassic north–south relative movement between Terrane I and these more easterly crustal rocks with which it collided. All the major faults in southeastern British Columbia west of the SRMT are cut by mid-Jurassic and/or Cretaceous batholiths, negating the possibility of any later major transcurrent movement. Examples occur at 125°30′ W, 55°30′ N; 120° W, 51°40′ N; 117°30′ W, 50°30′ N and 117° W, 49°30′ N. It seems that crustal rocks to which Terrane I became accreted in the mid-Jurassic have remained accreted to Terrane I ever since; it follows that the degree of north–south allochthoneity of these rocks is at least as great as that of Terrane I since the mid-Jurassic.

We propose the name 'Terrane III' for those crustal rocks which became accreted to Terrane I in the Jurassic; we show its approximate boundaries in Fig 1. In general, Terrane III is bounded to the west by Terrane I and to the east by the North American craton. Figure 1 shows the Purcell thrust as the general

eastern limit of Terrane III at the present erosion level, but it is possible that it may lie further east or west. In some locations, small klippen of Terrane I occur in Terrane III and inliers of Terrane III are found in Terrane I as a result of the mid-Jurassic collision. In places, klippen of Terranes I and III have been thrust onto rocks of the North American continent but these, we suggest, were emplaced at a later time (~100 Myr) when Terranes I and III together collided with the North American craton.

Cordilleria

As shown earlier, the available palaeomagnetic evidence indicates that the mid-Cretaceous position of Terranes I and II was 14–20° of latitude south of their present position relative to the North American craton and rotated 45–50° anticlockwise of their present orientation. The Cretaceous batholiths sampled for palaeomagnetic measurements on Terrane II are from the coast plutonic complex, the welt which formed during the accretion of Terrane II to Terrane I. As Terrane III has remained accreted to Terrane I since the Jurassic, it seems probable that Terranes I, II and III were all at least loosely amalgamated and at the latitude of present-day California in early to mid-Cretaceous times.

We propose the name 'Cordilleria' for this amalgamated super-terrane or microcontinent (Fig. 4). The southern limit of Cordilleria may be at or just south of the Lewis and Clark line in the northwestern United States, although it is possible that many or all of the suspect terranes of Oregon, Washington and California were joined formerly to Cordilleria. The northern limit of Cordilleria is not clear. Wrangellia, an element of Terrane II, was amalgamated with the Peninsular terrane of southern Alaska by mid-Jurassic times, forming the Talkeetna super-terrane[50]. The Peninsula and Kuskokwim terranes have been at the same latitude as Talkeetna since the mid-Jurassic[51] and Talkeetna was accreted to the Nixon Fork terrane and to the Yukon–Tanana terrane (an element of Terrane I) in the mid-Cretaceous[50]. This implies that a large portion of southern Alaska may have been included in the microcontinent Cordilleria.

Cretaceous subduction

Figure 5 shows one possible sequence of events for the movement of Cordilleria during the Cretaceous and here we describe a model which we believe best fits the evidence. Accurate dates are difficult to obtain because most of the available data are in the form of K–Ar ages. These tend to be minima, dating the time of final uplift and cooling of a terrane, and are also subject to problems of argon loss (and gain) and atmospheric contamination. Fitting the palaeomagnetic data into these ages causes further difficulties because palaeomagnetic blocking temperatures tend to be higher than K–Ar blocking temperatures. Further, there can be a time lag as long as 10 Myr between the onset of subduction and the K–Ar age given by a generated pluton. Present-day plate boundaries change on average every 10 Myr and every 500 km. Hence it is unreasonable to expect past plate boundaries to have been longer lasting or more continuous in type along strike.

Despite these uncertainties, it seems from the palaeomagnetic evidence that Cordilleria, relative to North America, has moved a distance of at least 1,500 km northwards since early to mid-Cretaceous. Elements of Cordilleria have rotated (possibly by internal deformation) ≤50° clockwise during the same period. Relative motion between Cordilleria and the North American craton was also affected by the westward movement of the North American plate as the North Atlantic opened.

In our model, the translation and rotation of Cordilleria is effected by seafloor spreading at the Kula–Farallon and the Kula–Pacific ridges, as well as by the northward motion of the Kula–Farallon–Pacific triple junction. Not all of the movement would have occurred along the boundaries of Cordilleria; some may have taken place within the microcontinent as the loosely

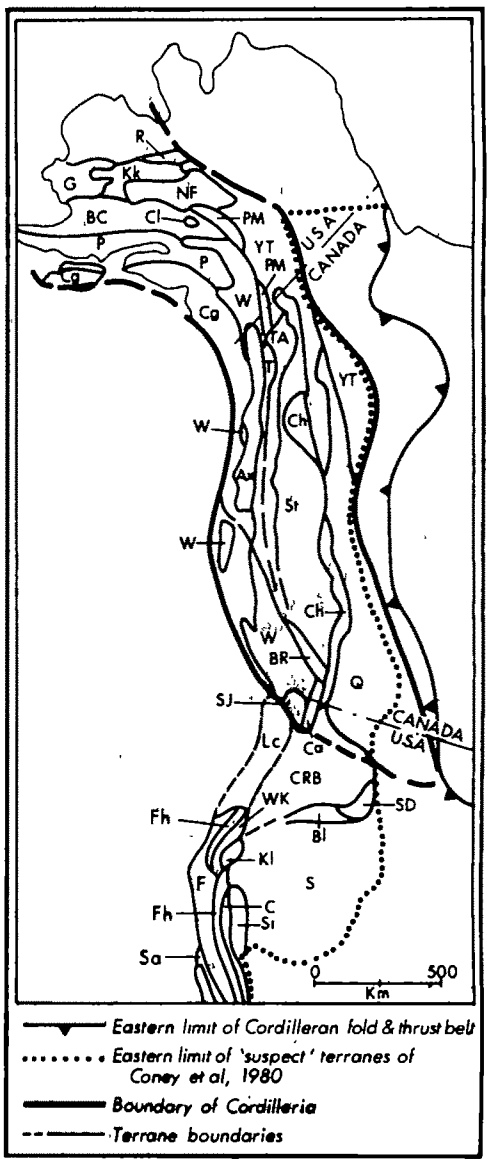


Fig. 4 The western Cordillera of North America, showing the terranes and the approximate boundaries of Cordilleria (after Coney *et al*[5] and Stone *et al*.[51]). Ax, Alexander, BC, Upper Mesozoic flysch; Bl, Blue Mountains, BR, Bridge River; C, Calaveras, Ca, northern Cascades, Cg, Chugach; Ch, Cache Creek; Cl, Chulitna; CRB, Columbia river basalts; F, Franciscan, Fh, foothills; G, Goodness; Kk, Kuskokwim; Kl, Eastern Klamath Mountains, LC, Lower Cenozoic volcanic and sedimentary rocks; NF, Nixon Fork; P, Peninsular, PM, Pingston and McKinley; Q, Quesnellia; R, Ruby, S, Sonomia, SD, Seven Devils; Si, northern Sierra; SJ, San Juan; St, Stikina; T, Taku, TA, Tracy Arm; W, Wrangellia; WK, Western Klamath Mountains; YT, Yukon–Tanana, Sa, Salina.

amalgamated terranes moved relative to each other. Deformation of continental lithosphere rarely occurs on a single fault[23].

The relative motion between Cordilleria and the craton could have been taken up by transcurrent faulting, or subduction, or both. The western margin of the North American craton at this time was approximately along the line of the Tintina, NRMT and SRMT faults. Transcurrent faulting has taken place along the Tintina/NRMT fault; there is little evidence for transcurrent movement on the SRMT and no evidence of transcurrent faulting to the south. Further, the total amount of documented movement on the northern faults is insufficient to account for all of the palaeomagnetically required motion. Hence, in our model, the relative motion between Cordilleria and the North American craton is taken up largely by southward and then south-westward-dipping oblique subduction of a late Jurassic back arc

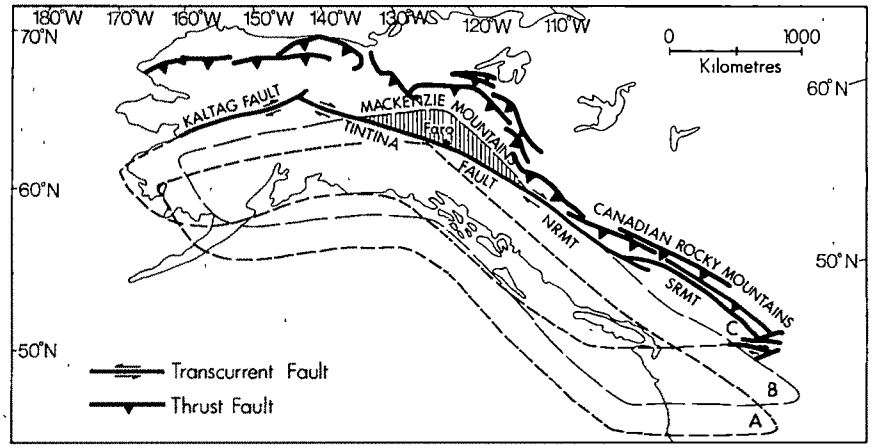


Fig. 5 Lambert equal area projection of the 'docking' of Cordilleria during the Cretaceous superimposed on the tectonic map of the present north-east Pacific (after Drummond *et al*[61]) A, B, C, Possible successive positions of the Cordilleria microcontinent as it collided with the North American craton. The shape of Cordilleria is speculative, approximating its present shape throughout, although it must have changed after collision. When in position A it was probably a looser amalgamation of Terranes I, II and III A possible protuberance which under- or overrode the North American craton at Faro is shown shaded in B. Other protuberances may also have existed, for example, one of Belt–Purcell strata (north of A) may have been thrust onto the craton when Cordilleria reached B, before transcurrent faulting moved the rest of the microcontinent into position C. Accretionary wedges forming above subduction zones along the northern and eastern margins of Cordilleria modified its shape as it moved northward. The collision at B was accompanied by tighter welding of Terranes I, II and III and a further generation of intrusives between Terranes I and II as subduction jumped westward. B represents a restoration of the 450 km of transcurrent motion on the Tintina fault and shows that the dextral transcurrent movement (B–C) would have been accompanied by an anticlockwise rotation of Cordilleria, thereby causing convergence in the Canadian Rocky Mountain region Since then, parts of Terrane II, the Pacific rim terranes and parts of southern Alaska have moved northwestward and westward on the Denali and other faults, further modifying the shape of Cordilleria.

basin beneath the northeastern margin of Cordilleria. The Cretaceous granitoids of the Omineca belt (with their relatively high strontium isotope initial ratio) could have been generated by subduction-related heating of the downgoing slab and the overlying relatively old crustal rocks of Terrane III. Volcanoes above these plutons could account for the first appearance of bentonites in the Alberta basin at ~100 Myr[52]. Subduction, besides taking up much of the palaeomagnetically required motion, would also provide a mechanism for Cretaceous (as opposed to Jurassic) thrusting and an accommodation at depth for the ~200 km of crustal shortening apparent in the southern Canadian Rocky Moutain thrust and fold belt[53]. An accretionary wedge building in the trench above this subduction zone produced the Mesozoic flysch terranes of southern Alaska[50], and further south it could have accumulated some of the $\sim 1 \times 10^6$ km^3 of material that has been eroded from the Omineca crystalline belt since the mid-Jurassic. If half of the detritus eroded during this unroofing of the Shuswap complex was later thrust onto the North American continent (under the Rocky Mountain thrust sheets) it could account for some of the ~20 km of very low-density material there[46] and for the location of the high negative Bouguer anomaly[45]. Graphite or pyritiferous black shales in this sedimentary pile could account for the very high electrical conductivity measured in this region (D. I. Gough, in preparation).

The first evidence of collision of Cordilleria with the North American craton seems to be the ~95 Myr plutons of the Mackenzie Mountains in the Yukon[54]. These plutons include S-type granites[55] consistent with generation by crustal thickening as the north-east corner of Cordilleria collided with the craton (Fig. 5, positions A–B). This is the only region where Cretaceous intrusive rocks and 'suspect' terranes occur east of the Tintina–Rocky Mountain Trench fault line. Certainly there has been obduction in this area[50] and a collision near Faro would be consistent with these facts as well as with the general shape of the Mackenzie Mountain thrust belt (Figs 5 and 6*a*).

Collision may also have occurred at about the same time along other protuberances of the Cretaceous North American craton; for example, the thrust slices of gneissic rocks east of the SRMT near Valemount and/or the Belt and Purcell groups in the northern United States (Fig. 6*b*, *c*). Extensions of Cordilleria north and south of point B in Fig. 5 may have broken off as the microcontinent collided with the North American craton at Idaho, the northern extension now forming the Belt group of the northwestern United states and the southern extension the Seven Devils arc and Blue Mountain terranes of Oregon and possibly even some of the suspect terranes of western California (Fig. 4)

The terranes of Cordilleria telescoped during collision and plate motions changed afterwards. The subduction zone jumped westward and changed polarity, initiating the late Cretaceous–early Tertiary plutonism of the coast ranges, and effecting a final suturing of Terranes I and II. Cordilleria was constrained to move northwestward and to rotate anticlockwise along the newly formed Tintina and NRMT transcurrent faults (Fig. 5, positions B–C). Transcurrent movement may have extended to the SRMT fault as well, but examination of Fig. 5 suggests that a small wedge-shaped basinal area along the SRMT would have closed slowly as Cordilleria rotated anticlockwise. This motion would rotate the southern end of the microcontinent over the craton, pushing a miogeosynclinal wedge of thrust sheets in front of it (Fig. 6*b*). Such a rotation would produce maximum (~200 km[53]) overthrusting at the southern end of the Canadian Rockies and minimum (~50 km[56]) overthrusting at the northern end. Meanwhile, the final collision of the northern end of Cordilleria with northern Alaska caused the ~200 km of overriding seen there[51] and the cataclasis of large portions of the northern Yukon–Tanana terrane. South of Cordilleria, the anticlockwise rotation may have been taken up by oblique northeasterly subduction of the oceanic part of the Kula plate under the North American craton producing the Idaho batholith (Fig. 6*c*). The radiometric ages obtained on the Idaho batholith[57] and the Valhalla dome[58] as well as the youngest ages obtained on the Proterozoic and Archaean metamorphic units along the SRMT[28] are mainly in the range 60–90 Myr. We suggest that these are intrusive or cooling ages marking the time of final suture of Cordilleria with North America, at ~80 Myr, a time when the rate of opening of the North Atlantic reached a maximum[59].

During the northward movement of Cordilleria and during and after its time of collision with the North American craton, further readjustments of the individual terranes took place; the Fraser, Denali and Queen Charlotte faults all record post-Cretaceous transcurrent movement. Evidence of late Tertiary extension is seen in normal faulting on many of the southern Cordilleria faults, including the SRMT.

The rate of northward movement of Cordilleria, relative to the North American craton, is diffiult to estimate because of the problem mentioned above of obtaining exact ages on intrusive rocks. The most likely dates are 120–110 Myr for a position ~1,500 km south of present and 105–100 Myr for position A (Fig. 5) which would require ~1,000 km of northward movement in 10–15 Myr at an average rate of 100–150 mm yr^{-1} (The remaining 500 km of northward movement were taken up by transcurrent movement in the north along the Tintina fault, and by oblique subduction and thrusting in the south.)

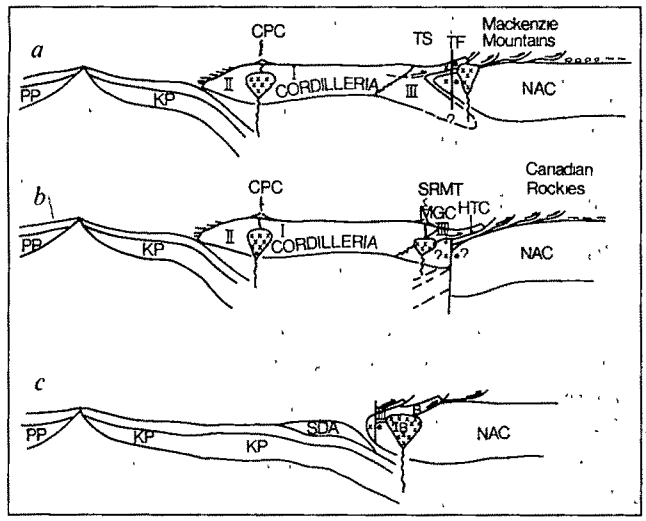


Fig. 6 Cross-sections at ~80 Myr drawn at three different latitudes. *a*, Section at the approximate latitude of Faro, Yukon, 62° N. *b*, Section at the latitude of the Malton gneiss complex near Valemount, BC 52°30′ N. *c*, Section at the approximate latitude of Salmon, Idaho 45° N. B, Belt group; CPC, coast plutonic complex; HtC, Horsethief Creek group; IB, Idaho batholith; KP, Kula plate; MGC, Malton gneiss complex; NAC, North American craton; PP, Pacific plate; SDA, Seven Devils arc; SRMT, Southern Rocky Mountain Trench; TF, Tintina fault; TS, Teslin suture. I, II and III indicate Terranes I, II and III. *a*, Westward-dipping subduction under Cordilleria during the early Cretaceous has closed a late Jurassic back arc basin between the North American craton and Cordilleria, causing a collision at ~100 Myr. The upper crust of Cordilleria was thrust onto the craton, carrying with it Terrane I ophiolites (Nisutlin, Simpson and Anvil allochthons) which had previously been obducted onto Terrane III during the mid-Jurassic collision of Terranes I and III along the Teslin suture. Crustal thickening due to the Cretaceous collision generated the S-type granites which invade the thrust sheets of the Mackenzie Mountains. After collision, motions changed, initiating dextral transcurrent movement on the Tintina fault, and subduction jumped westward, generating the granitoids of the coast plutonic complex and causing a final suturing of Terrane II to Terrane I. *b*, Early Cretaceous westward-dipping subduction which generated granitoids along the eastern edge of Cordilleria (Murtle stock at this latitude) ceased with the collision of Cordilleria and the North American craton along the line of the SRMT. The main collision at this latitude was later than in *a* because it was caused by the anticlockwise rotation of Cordilleria as it moved along the Tintina fault. During the collision, supracrustals from Cordilleria (Horsethief Creek, Kaza) were thrust onto the craton covering the suture (SRMT) between the basement blocks. Subduction jumped westward as in *a*. *c*, The first collision occurred at about the same time as in *a*, thrusting supracrustals from Cordilleria (belt group) onto the North American craton. After the collision, Cordilleria, carried on the Kula plate, moved northwestward ~450 km, rotating anticlockwise. The northwestward translation moved the southern margin of Cordilleria into a position north of Salmon; the Seven Devils arc may be a remnant of Cordilleria, broken off as the main body of the microcontinent moved northwestward. The anticlockwise rotation caused the southern end of the Kula plate to swing northeastward, the motion being taken up by its subduction under the North American plate, generating the Idaho batholith.

We propose that Cordilleria was carried northwards on the Kula plate as it moved away from the Kula–Farallon rift opening to the south. The calculated rate of movement of the Kula with respect to the American plate, obtained by extrapolating magnetic measurements back to the beginning of the Cenozoic, is 120 mm yr^{-1} northwards[60], a value quite close to the rate of movement suggested here. At the same time, the Kula–Pacific ridge was spreading and the American plate was moving westwards as the North Atlantic opened.

Conclusions

The increasing and consistent palaeomagnetic data on Mesozoic igneous rocks of south-central British Columbia places them at the latitude of California and rotated 45° anticlockwise from their present orientation. Existing theories do not include any line of suture between the Intermontane belt and the Rockies along which Mesozoic or early Tertiary northward movement and closure could have occurred. Our investigations show that the Malton gneiss complex (52°20′ N on the SRMT) is a composite feature, a window into a basement which differs on either side of the SRMT. Noting that all geophysical parameters change across the SRMT, we consider that it marks a line of weakness above a Cretaceous suture, now largely covered by the thrust sheets of the Rockies.

All the evidence can be reconciled if a newly defined microcontinent, Cordilleria, corresponding to most of British Columbia and part of the Yukon and Alaska and perhaps parts of Oregon and California, existed. This microcontinent must have lain on the Kula plate and west of a subduction zone, along which convergence occurred during the late Jurassic and early Cretaceous, closing a back-arc basin and bringing Cordilleria northward as well as towards the craton. The latter overrode the trench and was struck by Cordilleria in the South Yukon causing the Mackenzie Mountains at ~100 Myr. Closure of the remaining oceanic gap between the craton and Cordilleria caused Rocky Mountain thrusting over the next 40 Myr. In our model, the Tintina–Rocky Mountain Trench system marks the line of the former trench system and its associated dextral faults. As the convergence was oblique, however, and much of the 1,500–2,000 km displacement was taken up by subduction, equivalent amounts of transcurrent movement will not have occurred along all parts of the suture. Any transcurrent faults generated would tend to grow at one or both ends (as with the present day San Andreas) with maximum relative faulting at the point of first collision.

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1 Monger, J W. H, Souther, J G & Gabrielse, H *Am. J Sci.* **272**, 577–602 (1972).
2 Monger, J. W H & Price, R A *Can J Earth Sci.* **16**, 770–791 (1979)
3 Davis, G A, Monger, J W H. & Burchfiel, B.C *Pacif. coast Paleogeogr Symp* **2**, 1–32 (1978)
4. Dickinson, W R *Can J Earth. Sci* **13**, 1268–1287 (1976).
5 Coney, P J., Jones, D L & Monger, J W. H *Nature* **288**, 329–333 (1980)
6 Tozer, E T *Geol. Surv Can econ Rep* **1**, 633–640 (1970)
7 Monger, J W H. & Ross, C A *Can J Earth Sci* **8**, 259–278 (1971).
8 Tipper, H W *Can. J Earth Sci.* **18**, 1788–1792 (1981).
9 Siberling, N J & Jones, D L *Geol Ass Can Abstr* **8**, A62 (1983)
10 Tozer, E T *Geol. Rdsch.* **71**, 1077–1104 (1982)
11 Packer, D R & Stone, D B *Can J Earth. Sci* **11**, 976–997 (1974)
12 Hillhouse, J W *Can J Earth Sci.* **14**, 2578–2592 (1977)
13 Van der Voo, R, Jones, M, Gromme, C S, Eberlein, G D. & Churkin, M *J geophys Res* **85**, 5281–5296 (1980)
14 Irving, E Monger, J W. H & Yole, R W *Geol Ass Can. spec. Pap* **20**, 441–455 (1981)
15 Irving, E, Wynne, P J. & Woodsworth, G J *Geol Ass Can. Abstr* **8**, A35 (1983)
16. Symons, D T A *Geol. Ass. Can Abstr* **9**, 109 (1984)
17. Monger, J W H, Price, R A & Tempelman-Kluit, D J *Geology* **10**, 70–75 (1982)
18. Gabrielse, H & Dodds, C J. *Geol. Ass Can Abstr* **2**, 19 (1977)
19 Templeman-Kluit, D J *Geol. Surv. Can Pap* **77-1A**, 223–227 (1977).
20 Leech, G B *Geol. Surv Can. Pap* **66-14**, 307–329 (1966)
21 Murphy, D C *Geol. Surv Can Pap* **84-1A**, 627–630 (1984).
22 Rogers, G C, Ellis, R M & Hasegawa, H S *Bull seism. Soc. Am.* **70**, 1771–1786 (1980)
23. McKenzie, D. & Jackson, J *Earth planet Sci Lett* **65**, 182–202 (1983)
24 Campbell, R B. *Geol Surv Can Map* **15-1967** (1968)
25 Chamberlain, V E, Lambert, R St J, Baadsgaard, H & Gale, N H *Geol Surv Can Pap* **79-1B**, 45–50 (1979)
26 Chamberlain, V E, Lambert, R St J. & Holland, J G *Precamb Res* **11**, 1–9 (1980)
27 Chamberlain, V E, Lambert, R St J & Holland, J G *Precamb Res* **11**, 297–306 (1980)
28 Chamberlain, V E thesis, Univ Alberta (1983)
29 Chamberlain, V. E, Lambert, R St J & Holland, J G *Can J Earth Sci.* (in the press)
30 Morrison, M L *Geol Surv Can Pap* **79-1B**, 407–410 (1979)
31 Oke, C & Simony, P S *Geol. Surv Can Pap* **81-1A**, 181–184 (1981)
32 McDonough, M R & Simony, P. S *Geol. Surv Can Pap* **84-1A**, 99–102 (1984)
33 Leonard, R. *Geol. Surv Can Pap* **8411A**, 121–127 (1984).
34 Coney, P J *Geol Soc Am Mem* **153**, 7–31 (1980)
35 Price, R A & Mountjoy, E W *Geol Ass Can Spec Pap* **6**, 7–25 (1970)
36 Ellis, R M & Chandra, B *Can J. Earth Sci* **18**, 1708–1716 (1981)
37 Mereu, R F, Majumdar, S C & White, R. E *Can J Earth Sci* **14**, 196–208 (1977)
38 Berry, M. J & Forsyth, D A *Can J Earth Sci* **8**, 788–801 (1975)
39. Wickens, A J *Can. J Earth Sci* **14**, 1100–1115 (1977)
40 Spence, G D, Clowes, R M & Ellis, R. M. *Bull seism. Soc Am.* **67**, 543–546 (1977)
41 Wickens, A J & Pec, K *Bull seism Soc. Am* **58**, 1821–1831 (1968).
42. Haines, G V, Hannaford, W & Riddihough, R P *Can J. Earth Sci* **8**, 387–391 (1971)
43 Dragert, H. & Clarke, G K C *J Geophys* **42**, 373–390 (1977)
44 Davis, E E & Lewis, T J *Can J Earth Sci* **21**, 715–726 (1984)

45. *Gravity Map Ser.* No. 74-1 (Department of Energy, Mines and Resources, Canada, 1974).
46. Chandra, N. N. & Cumming, G. L. *Can. J. Earth Sci.* **9**, 1099–1109 (1972).
47. Leggett, J. K., McKerrow, W. S. & Soper, N. J. *Tectonics* **2**, 187–210 (1983).
48. Brown, R. L. & Murphy, D. C. *Can. J. Earth Sci.* **19**, 456–465 (1982).
49. Templeman-Kluit, D. J. *Geol. Surv. Can. Pap.* **79-14**, 27 (1979).
50. Csejty, B., Cos, D. P., Evarts, R. C., Stricker, G. D. & Foster, H. L. *J. geophys. Res.* **87**, 3741–3754 (1982).
51. Stone, D. B., Panuska, B. C. & Packer, D. R. *J. geophys. Res.* **87**, 3697–3707 (1982).
52. Spears, D. A. & Duff, P. *Can. J. Earth Sci.* **21**, 465–476 (1984).
53. Price, R. A. in *Thrust and Nappe Tectonics* Vol. 9 (eds MacClay, K. & Price, N. J.) 427–448 (Geol. Soc. Lond. spec. Publ., 1981).
54. Anderson, R. G., Armstrong, R. L., Parrish, R. & Bowmam, J. R. *Geol. Ass. Can. Abstr.* **8**, A2 (1983).
55. Anderson, R. G. *Geol. Ass. Can. Abstr.* **8**, A2 (1983).
56. Thompson, R. I. in *Thrust and Nappe Tectonics* Vol. 9 (eds MacClay, K. & Price, N. J.) 449–462 (Geol. Soc. Lond. spec. Publ., 1981).
57. Armstrong, R. L., Taubeneck, W. H. & Hales, P. O. *Bull. geol. Soc. Am.* **88**, 397–411 (1977).
58. Parrish, R. & Ryan, B. *Geol. Ass. Can. Abstr.* **8**, A53 (1983).
59. Briden, J. C., Hurley, A. M. & Smith, A. G. *J. geophys. Res.* **86**, 11631–11656 (1981).
60. Atwater, T. *Bull. geol. Soc. Am.* **81**, 3513–3536 (1970).
61. Drummond, K. J., Nishiwaki, C., Corvalan, J., Doutch, H. F. & Craddocks, C. *Am. Ass. Petrol Geol. Tectonic Map* (1982).

Structural analysis of murine genes containing homoeo box sequences and their expression in embryonal carcinoma cells

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The presence of homoeo box sequences in the genomes of vertebrates has suggested that these metazoans possess the equivalent of the homoeotic genes that have a key role in regulating the development of the fruitfly Drosophila melanogaster. *We report here that a novel murine homoeo box-containing gene is expressed in embryonal carcinoma stem cells. Transcripts of the sequences that flank the homoeo box of this gene are found in these cells before and after induced differentiation, whereas a specific transcript that seems to contain the homoeo sequence is only present after differentiation.*

THE establishment of embryonal carcinoma (EC) cell lines has facilitated greatly the study of early stages in mouse embryogenesis. EC cells are derived from the pluripotent stem cells of teratocarcinomas[1] and possess many properties of ectodermal cells of the inner cell mass[2]. Advantages of these cells include the existence of various EC lines capable of differentiating into several tissue types *in vitro*, including extraembryonal endoderm, as well as their parallel use for *in vivo* studies[2]. One of these EC stem-cell lines, F9, was isolated from a transplanted male embryo[3] and expresses many antigens that are ascribed generally to embryonal carcinoma cells and mouse developing embryos[4–7]. F9 cells, unlike many other EC stem-cell lines, give rise spontaneously to differentiated cells at a very low rate[8], although differentiation can be induced by retinoic acid in the presence or absence of dibutyryl cyclic (c)AMP[9–11] or by transfection with c-*fos* proto-oncogenes[12]. The induced phenotypic alterations of retinoic acid-differentiated F9 cells are reflected in the production of markers characteristic of either visceral endoderm[10–13] or parietal endoderm[9,13–15], depending on the culture conditions. These extraembryonal tissues derive before implantation, during embryonal development, from a common stem-cell population, primitive endoderm.

Until recently the isolation of mouse genes expressed during development relied mainly on the identification of genetic information which is expressed discriminately in undifferentiated or differentiated EC cells[16,17]. In turn, we have used an alternative approach to attempt to isolate genes expressed during mouse development. This approach is based on the assumption that certain mouse developmental processes might also require the expression of homoeotic genes, similar to those of the fruitfly *Drosophila melanogaster*. To this end we screened mouse genomic information with *Drosophila* homoeo box sequences. These sequences (~200 base pairs, bp) are conserved in many of the developmental genes located in the antennapedia (ANT-C) and bithorax complexes of *Drosophila*[18–21]. These regions share a high degree of conservation not only in *Drosophila* but also with homoeo boxes found in human[22], murine[23] and *Xenopus*[24,25] DNA. Most impressive, however, is the fact that at least two of these genes have been isolated exclusively by their homology to *Drosophila* homoeo boxes and are transcribed in oocytes or during early embryogenesis in *Xenopus*[24,25]. Using two *Drosophila* probes we have isolated murine DNA sharing homology with previously described homoeo box sequences. This murine DNA detects the expression of sequences next to the homoeo box in EC stem cells as well as an additional RNA species that contains homoeo box sequences and accumulates during the differentiation of these cells. All these transcripts are encoded by the same DNA strand and are not detected in RNA isolated from macrophages.

Isolation of murine homoeo box sequences

At least eight *Eco*RI fragments of murine DNA hybridize with *Drosophila* homoeo box probes[19]. To isolate mouse homoeo box sequences, $\sim 3 \times 10^5$ members (~ 1 genome equivalent) of a mouse genomic library (gift of H. Lehrach) in λEMBL3 (ref. 26) were screened under conditions of reduced stringency using purified inserts of p903G, the 3′ region of a cDNA clone of *Antennapedia* (*Antp*)[18,27], and pFS2, the 3′ region of a genomic clone of *fushi tarazu* (*ftz*)[28]. Homologies between these two probes are confined to the sequences contained in their homoeo boxes. Of the recombinant phage screened, 10 recombinants were isolated, based on their hybridization to both probes. The DNAs of representative phage, cleaved with *Eco*RI, are shown in Fig. 1*a*. Hybridization of *ftz* and *Antp* probes to Southern blots[29] of these cleaved DNAs shows isolate-specific as well as probe-specific patterns. For example, λm5 and λm12 share two common fragments that hybridize similarly to both *Antp* and *ftz* probes, but λm6 shows hybridization of three fragments with the *ftz* probe while only two fragments hybridize to the *Antp* probe. As a control we used λm9, which is isolated by hybridization to the *ftz* probe alone. Hybridization of its *Eco*RI fragments confirms that this phage DNA hybridizes exclusively to *ftz* probe.

To determine which of the various fragments in λm5 and λm6 were hybridizing exclusively to homoeo boxes and which to flanking sequences contained within the probes, smaller fragments from *ftz* and *Antp* containing almost exclusively homoeo box sequences were hybridized with the cleaved recombinant DNA (Fig. 1*b*). λm5 and λm6 DNA showed hybridization of one and two *Eco*RI fragments, respectively, with both probes. The additional very faint band in λm5 which hybridizes with *Antp* is a partial digestion product and has not been otherwise observed. One of these recombinants, λm6, was mapped in

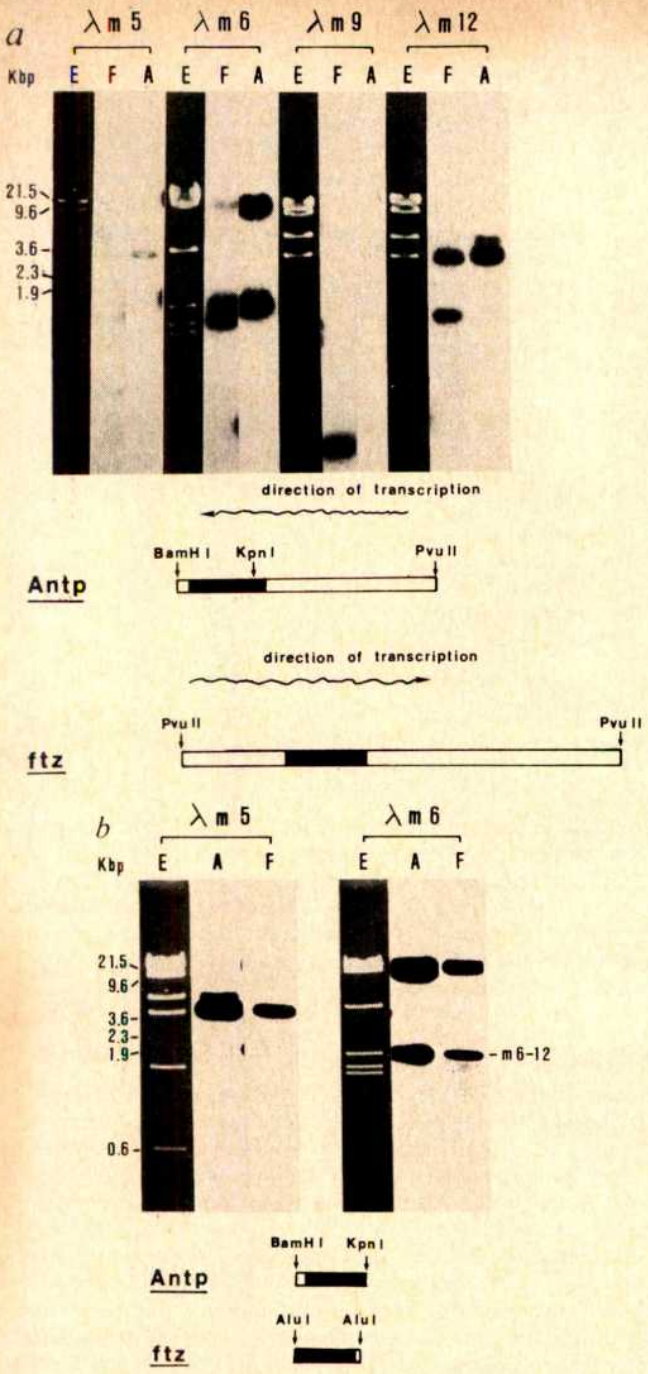


Fig. 1 Hybridization of inserts containing *Drosophila* homoeo boxes to λ mouse recombinant DNAs. *a*, Hybridization of λ mouse recombinants (λm5, 6, 9 and 12) DNAs to purified inserts of p903G (*Bam*HI/*Pvu*II) or pFS2 (*Pvu*II/*Pvu*II). Lanes E, ethidium bromide staining; F, *ftz* probe; A, *Antp* probe. *b*, Hybridization of *Drosophila* homoeo boxes to murine DNA. Phage DNAs were cleaved with *Eco*RI, separated on agarose gels (E), blotted onto nitrocellulose and hybridized with probes from *ftz* (F) or *Antp* (A). The structure of the probes and the homoeo box sequences therein (closed box) are represented beneath each panel.

Methods. Phage DNAs were isolated as described previously[40], cleaved with *Eco*RI and separated by electrophoresis in an agarose gel. Southern blots of the phage DNAs were hybridized initially with either *a*, the ^{32}P-labelled *Bam*HI/*Pvu*II fragment of p903G that contains a *Drosophila* homoeo box, then exposed, boiled in H_2O for 1 min to remove the probe and rehybridized to the homoeo box insert (*Pvu*II/*Pvu*II) of pFS2; or *b*, the nick-translated *Bam*HI/*Kpn*I insert of p903G (A) and an *Alu*I restriction fragment of pFS2 (F). The hybridizations were performed under conditions of reduced stringency (43% formamide, 5×SSC, 5×Denhardt's, 100–250 μg ml^{-1} denatured salmon sperm DNA, 50 mM Na_3PO_4, *p*H 7.0, 0.1% SDS, 37 °C) as described by McGinnis *et al.*[19] with washes at 50–55 °C in 2×SSC, 0.1% SDS.

detail and, based on overlapping restriction, hybridization patterns (Fig. 2) and subsequent sequence data, found to contain two homoeo box regions lying within 10 kilobases (kb) of one another on a contiguous stretch of murine DNA.

Murine homoeo box sequences

The hybridizing 1.9-kb *Eco*RI fragment of λm6, m6-12, was isolated subsequently and subcloned into pSP65. Finer mapping of this subcloned fragment showed that the homoeo box homologous region is confined to a 206-bp *Pvu*II/*Eco*RI fragment (Fig. 2). The sequence of this region, as well as the adjacent 300-bp *Eco*RI fragment, was obtained along both strands using chain termination[30] and chemical modification[31] techniques (Fig. 3*a*). Comparison of this sequence with the sequences of *Drosophila* (*Antp*, *ftz* and *Ubx*)[19], *Xenopus* (MM3)[25], murine (Mo-10)[23] and human (*Hu-1* and *Hu-2*)[22] homoeo boxes indicated striking, albeit varying, degrees of homology. The sequence m6 contains 83% homology to the homoeo boxes of *Hu-1* and *Hu-2*, whereas comparison with Mo-10 surprisingly showed only 78% homology; m6 is 84 and 82% homologous to *Antp* and MM3, respectively, although only 79 and 76% homologous to the other *Drosophila* homoeo boxes, *Ubx* and *ftz*, respectively.

Conceptual translation of the sequence obtained and comparison with the published homoeo box sequences of MM3, *Antp*, Mo-10, *Hu-1*, *Hu-2*, *ftz* and *Ubx* (refs 19, 22, 23, 25) are shown in Fig. 3*b*. The homologies in the amino-acid sequences were higher than those obtained with the nucleotide sequence because most nucleotide differences occur at the wobble position, indicating that the basis for the conservation of this region probably occurs at the protein level. The most homologous sequence showed the following homologies: to *Antp* (95%), *Xenopus* (93%), *Hu-2* (88%), *Hu-1* (85%), *Ubx* (85%) and *ftz* (80%). Surprisingly, the least homology in amino-acid sequence was seen with the mouse (Mo-10) homoeo box (72%). These sequences in the mouse seem to be the most divergent. We also observed at the end of the conserved homoeo box domain a succession of glutamic acid residues followed by a stop codon. As this nucleotide sequence was obtained along both strands using both sequencing methods, we are confident of its validity. A similar succession of acidic residues followed by an in-frame stop codon was observed by Müller *et al.*[25] in *Xenopus* and is also present in the sequence of *Hu-2* (ref. 22).

Expression of murine DNA

To determine whether the probe used for expression studies contained repetitive DNA, m6-12 DNA was hybridized with genomic blots of murine DNA (Fig. 4). DNA from F9 and another EC cell line, P19 (ref. 32), was cleaved with *Eco*RI, *Hin*dIII or *Kpn*I. Hybridization of m6-12 DNA with the cleaved DNAs under stringent conditions revealed hybridization to a single band in each case, showing that the sequences in m6-12 DNA detect a single-copy region. The expression of the murine DNA sequences containing homoeo box sequences and those adjacent to them was examined in three teratocarcinoma stem-cell lines. RNA was isolated from EK[33] cells and from various EC cell lines (PC13, P19 and F9 cells) and hybridized to plasmid DNA containing m6-12 (Fig. 5*a*). Two predominant bands appear in the RNAs from EK, PC13, P19 and F9 stem cells although in greatly varying amounts. The size of the larger and less abundant RNA is ~2.9 kb and the smaller RNA 1.5 kb. The quantities of poly(A$^+$) RNA from PC13, P19 and F9 blotted were corroborated by hybridization to β-actin. We observed quantitative differences in the amount of the 2.9- and 1.5-kb RNAs in the various stem-cell lines. There is 3–6 times more of the 1.5-kb messenger RNA in the F9 cells than in PC13 and P19 cells and ~12 times more of this transcript in F9 cells than in EK cells. The larger 2.9-kb mRNA is also more abundant in F9 cells compared with P19, PC13 and EK cells (an ~3.5 to 5-fold difference). These RNAs are ~10 times less abundant in F9 cells than β-actin RNA. These differences were normalized

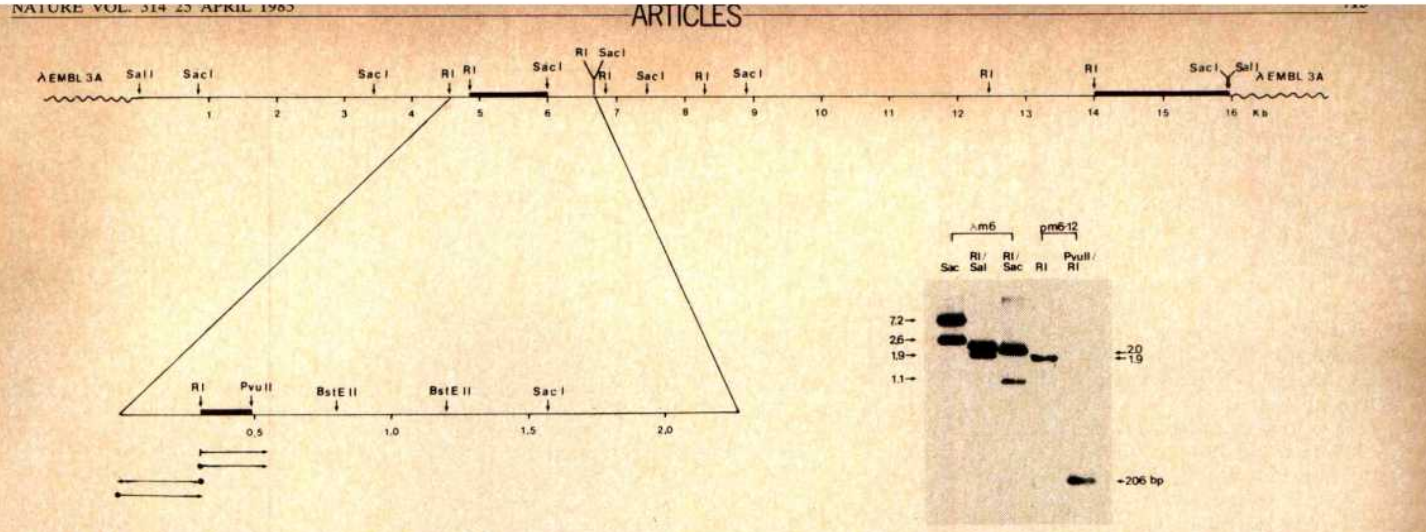


Fig. 2 Restriction mapping and subcloning of fragments contained in λ clone m6. The recombinant phage DNA contains *Mbo*I partially digested (~16 kb) murine liver DNA cloned into *Bam*HI sites of λEMBL3A (ref. 26). The figure localizes the homoeo box-homologous regions to 1.3-kb *Eco*RI/*Sac*I and 2.0-kb *Eco*RI/*Sal*I fragments (closed boxes).

Methods. Hybridizations used the 600-bp *Antp* cDNA fragment (see Fig. 1*a*) as probe. A 1.9-kb *Eco*RI fragment containing the 1.3-kb *Sac*I/*Eco*RI homoeo box-hybridizing fragment was subcloned into pSP65 to give pm6-12. The homoeo box homology was further confined in pm6-12 to a 206-bp *Pvu*II/*Eco*RI subfragment (see insert). The *Bst*EII/*Eco*RI fragment containing the *Pvu*II site was purified subsequently, 3′ end labelled at the *Eco*RI site with Klenow fragment and sequenced according to the method of Maxam and Gilbert[31]. This sequence was confirmed along the opposite strand by the method of Sanger *et al.*[30] using a subclone of the 1.3-kb *Sac*I/*Eco*RI fragment, mp11/m6-12. The adjacent 300-bp *Eco*RI fragment was cloned in both orientations in M13mp11 to obtain sequence information along both strands.

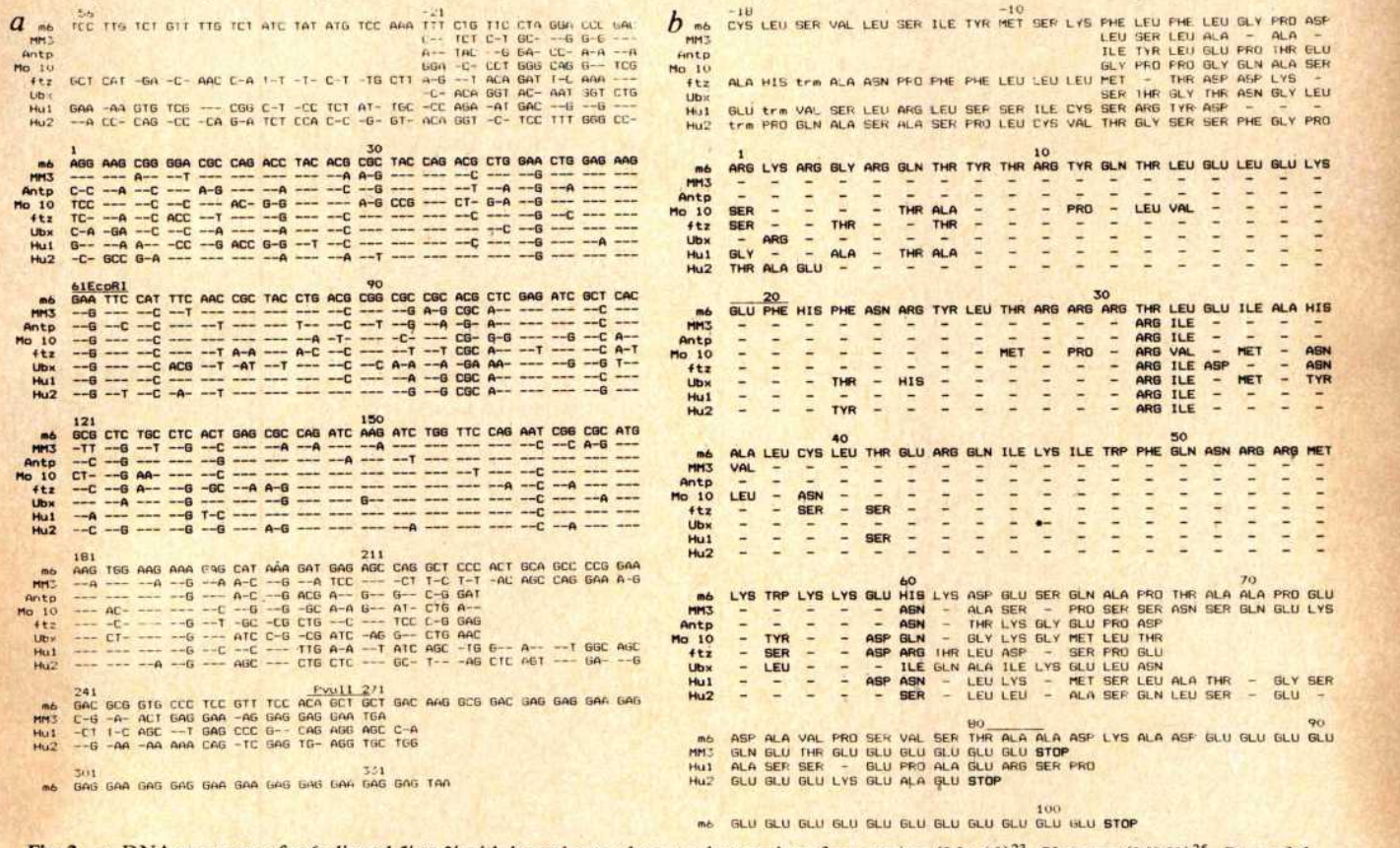


Fig. 3 *a*, DNA sequence of m6 aligned 5′ to 3′ with homologous homoeo box regions from mouse (Mo-10)[23], *Xenopus* (MM3)[25], *Drosophila* (*Antp*, *ftz*, *Ubx*)[19] and human (*Hu-1* and *Hu-2*)[22] DNA. Only those nucleotides that differ from m6 are shown. Conserved nucleotides are indicated by -. *b*, Conceptual translation of the conserved homologous open reading frame in m6 and its comparison with other homoeo box amino-acid domains. Comparison is with amino acids −18 to 83 as numbered in refs 19, 22, 23 and 25. Only non-homologous amino acids are shown directly; conserved amino acids are indicated by -; the asterisk indicates a change in the published amino-acid sequence of *Ubx* (W. Gehring, personal communication).

relative to the amount of β-actin mRNA in each sample as evidenced by hybridization of the blot with a β-actin probe.

To look for RNA encoding potential regulatory proteins induced during differentiation, F9 cells were differentiated by treatment with 5×10^{-7} M retinoic acid and 10^{-3} M dibutyryl cAMP. Poly(A)$^+$ RNA was obtained at different times during the first 24 h of differentiation and after 5 days of treatment (Fig. 5*b*). At 17 h, at least one new RNA (2.4 kb) appeared, although longer exposures of the blot show the presence of an additional low-level 3.2-kb transcript that shows a marginal induction on differentiation of the F9 cells (data not shown). The other two transcripts present in undifferentiated F9 cells, the 1.5- and 2.9-kb mRNAs, remain present during the differenti-

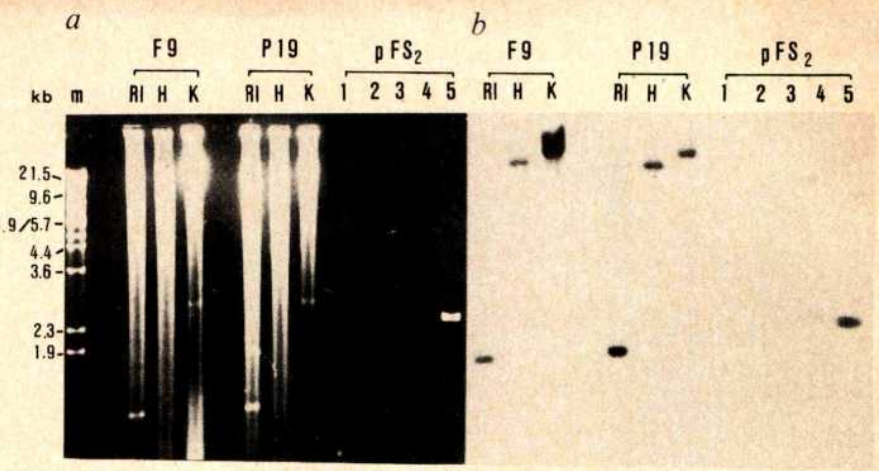


Fig. 4 Hybridization of murine DNA containing a homoeo box (m6-12) to mouse genomic DNA from F9 and P19 cells. *a*, Ethidium bromide staining of cellular DNA. Cell DNA was isolated by treatment of cells with lysis buffer (50 mM Tris-HCl, 20 mM EDTA, 100 mM NaCl, *p*H 7.5) and digestion with proteinase K (50 μg ml^{-1}) for 16–24 h. DNAs were purified by phenol extraction and ethanol precipitation. DNAs were cleaved with *Eco*RI (RI), *Hin*dIII (H) or *Kpn*I (K) and separated on a 0.7% agarose gel (*b*). A Southern blot of DNA in gel *a* was hybridized with nick-translated (m6-12) DNA (1.9 kb *Eco*RI fragment). Various concentrations of uncleaved pFS2 DNA (1, 0.01 ng; 2, 0.1 ng; 3, 1 ng; 4, 10 ng; 5, 100 ng) were included. Hybridization, under stringent conditions, used the same buffer as in ref. 19, with 50% formamide. The blots were washed at least four times in 0.1 × SSC and 0.1% SDS at 52 °C for 15 min each.

ation of the F9 cells, although one of these (2.9 kb) shows a slight increase (~twofold) during this period.

To determine whether these mRNAs were present 5 days after induction of differentiation, poly(A$^+$) RNA from F9 cells treated with retinoic acid and dibutyryl cAMP for short (< 24 h) and long (5 days) periods were compared. All the transcripts that hybridized with m6-12 at 17 and 24 h (3.2, 2.9, 2.4 and 1.5 kb) were also present at 5 days, although in apparently reduced quantities (1.5–6 times less) compared with the earlier times. Each value was once again standardized by comparison with the amount of poly(A$^+$) RNA blotted for each sample, as determined by β-actin hybridization.

The presence of either homoeo box or flanking sequences in each of these predominant RNA species was examined by hybridization with subfragments contained in the 1.9-kb *Eco*RI fragment, m6-12, which had been used in the initial Northern analyses. Blots of poly(A$^+$) RNAs from stem and retinoic acid- and dibutyryl cAMP-differentiated (24 h) F9 cells were hybridized with either the *Eco*RI/*Pvu*II 1.7-kb fragment containing flanking sequences (Fig. 6*a*) or the 0.206-kb *Pvu*II/*Eco*RI fragment that largely contains the murine homoeo box sequences (Fig. 6*b*). The flanking sequences hybridize with all of the transcripts previously seen in F9 cells at 17 h, 24 h and 5 days after treatment with retinoic acid and dibutyryl cAMP. The probe containing the homoeo box, however, hybridizes strongly to only one of these mRNAs, the 2.4-kb mRNA, which is induced shortly after differentiation. In addition, there is a weak hybridization of this probe to the most abundant of these mRNAs, the 1.5-kb species. To determine whether the observed transcripts are encoded by the same or the opposite strand of m6-12 DNA, single-stranded probes were used (Fig. 6*c*). Hybridization of the noncoding (panel 1) and coding (panel 2) strands showed that all the RNAs detected by the m6-12 (panel 3) probe are complementary to the coding strand and are thus transcribed from the strand encoding the conceptually translated homoeo box open reading frame.

Conclusions

One of the central aims of developmental biology is to understand the molecular details of the mechanisms governing the precise control of differentiation processes. Although little is known about the regulatory mechanisms of mammalian development, the wealth of genetic information[34] and the availability of EC stem-cell lines led us to choose these as model systems for the study of mammalian development. Differentiation of F9 stem cells is possible either by treatment with retinoic acid or by transfection with an oncogene (c-*fos*). It remains unclear, however, whether this results from a direct or indirect action of the inducer.

Discovery of common homoeo box sequences in many of the developmental genes in *Drosophila* and developmentally regulated genes of *Xenopus* led us to isolate analogous genes and to study their products in murine EC cells. So far, we have isolated seven mouse phage recombinants that contain homology to the *Drosophila* homoeo boxes and show different restriction patterns. Detailed examination of one λm clone (λm6) indicated the presence of at least two homoeo box regions.

Analysis of the nucleotide sequence of one of these regions revealed an extremely well-conserved sequence of 180 bp. Approximately 84% homology to *Antp* can be detected at the nucleotide sequence level. The amino-acid sequences of these conceptually translated proteins are even more highly conserved (95% homology). Amino-acid homology with proteins of known structure (cro and *c*I) suggests that the most conserved region includes an α-helical domain (ref. 20 and W. Gehring, personal communication). Interestingly, the most homology was to the homeo boxes of the *Antp* gene of *Drosophila*, *Xenopus* (MM3) and the human genome, whereas the least homology was to the murine Mo-10 gene. Whether this structural resemblance to the *Antp* gene, MM3 and the human genes also reflects a functional resemblance remains to be determined. Clearly, the m6 homoeo box region is different from the Mo-10 gene. Further comparison of MM3 with m6 reveals conservation of both the homoeo box and a stretch of glutamic acid residues, which lies outside the homoeo box and at the carboxy-terminus of the putative protein. Although the functional significance of this acidic domain remains necessarily speculative, such domains exist in the non-histone nucleosomal proteins HMG1 and HMG2 (ref. 35) and in a neurofilament protein, NF-M (ref. 36).

Before using m6-12 sequences as a probe for Northern analyses, they were characterized by hybridization to genomic blots of murine DNA. Although the m6-12 DNA contains homoeo box sequences present in several copies in the murine genome, Southern analysis showed that it hybridized to unique DNA. The inability of this probe, which primarily contains flanking sequences, to detect other homoeo boxes in the murine genome, may have resulted from the very stringent conditions used in the Southern hybridizations in contrast to the non-stringent conditions of the library screening. Furthermore, possible differences in the sequences of murine homoeo boxes (compare m6 and Mo-10 sequence homology) and the specific activity of homoeo box sequences contained in the probe (< 7%) could have precluded their detection.

The probe containing murine homoeo box sequences was used for studies of RNA expression in EK and various stem cells. Surprisingly, F9 stem cells show a strong expression of two differently sized classes of RNA. The same RNA classes can also be seen in EK, PC13 and P19 cells, although in reduced amounts. One interpretation of these results is that F9 cells are already pre-determined and do not represent a true stem cell any longer. Similar conclusions have been drawn by Adamson and Hogan[37] on the basis of low-level synthesis of transferrin

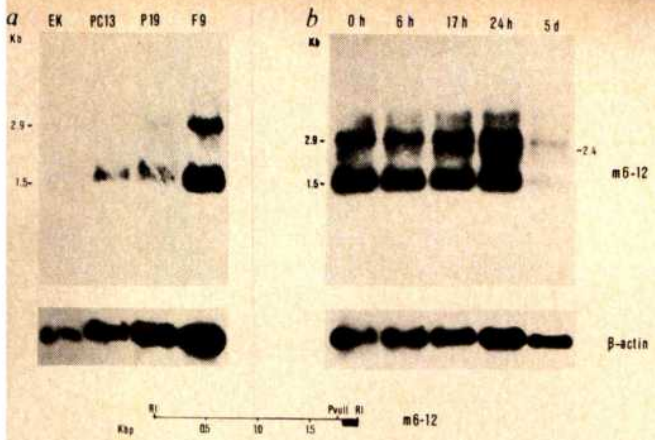


Fig. 5 Hybridization of m6-12 DNA with mRNA from various teratocarcinoma stem cells and differentiated F9 cells. *a*, Poly(A^+) RNAs from EK, PC13, P19 and F9 cells were hybridized with ^{32}P-labelled pm6-12 DNA. *b*, RNA obtained from F9 cells treated with retinoic acid (5×10^{-7} M) and dibutyryl cAMP (10^{-3} M) for 6, 17, 24 h and every other day for 5 days were hybridized with ^{32}P-labelled pm6-12 DNA. Autoradiograms of control hybridizations of each Northern blot (*a* and *b*) with ^{32}P-β-actin DNA are shown below each corresponding panel. Differences in the intensities of the bands were quantitated by densitometer scanning. The structure of the m6-12 insert in the probe is shown at the bottom of the figure; the solid box represents the homoeo box sequence.

Methods. RNA samples were isolated using the guanidinium thiocyanate method of Chirgwin *et al.*[41] and purified by centrifugation through a 5.7 M CsCl, 25 mM sodium acetate, *p*H 5.0 cushion. Poly(A^+) RNAs were obtained from each group by retention on oligo(dT) columns, then 5 μg of poly(A^+) RNAs was placed in each well and electrophoresed through formaldehyde–agarose gels using 3.7% formaldehyde in MOPS buffer (20 mM morpholinopropane sulphonic acid, 50 mM sodium acetate, 10 mM EDTA *p*H 7.0). RNAs were blotted onto Gene Screen Plus and hybridized to nick-translated plasmid containing m6-12 DNA (lower portion of the figure) and then to β-actin DNA. Briefly, Northern blots were hybridized with ^{32}P-DNA in 50% formamide, 1.0 M NaCl, 1% SDS and 100 μg ml^{-1} denatured salmon sperm DNA. Blots were washed twice in 2×SSC at room temperature, twice in 2×SSC, 1% SDS at 60°C, and twice in 0.1×SSC at room temperature.

and expression of epidermal growth factor receptors in F9 stem cells, markers which are not usually present on other stem cells.

We were also interested to determine whether differentiation of F9 cells is accompanied by the differential expression of murine genes. RNA produced by F9 cells at various times after differentiation into parietal endoderm showed the early appearance of an additional transcript (2.4 kb) that hybridized with both the homoeo box and flanking sequences contained in the m6-12 probe. The transcripts present in F9 stem cells and those which increase so rapidly during the first 24 h of differentiation persist well into the time when F9 cells express markers of differentiated cells (5 days), although in decreased quantities compared with another transcript such as β-actin. RNA from macrophage, a terminally differentiated cell type, did not accumulate detectable amounts of these mRNAs (data not shown). To establish the relationship between the detected RNAs, the 206-bp *Pvu*II/*Eco*RI fragment containing the murine homoeo box and an additional 90 bp was hybridized with mRNAs from F9 stem and differentiated (24 h) cells and the 2.4-kb mRNA was detected, suggesting that the transcript contains homoeo box sequences. Although there is also a weak hybridization to the most abundant mRNA (1.5 kb; data not shown), the intensity of this hybridization, compared with that seen with the 2.4-kb mRNA, suggests that not all of the probe sequences are present in this transcript. This hybridization could result either from homology with the 206-bp probe or from a minor contamination of this probe with the larger 1.7-kb

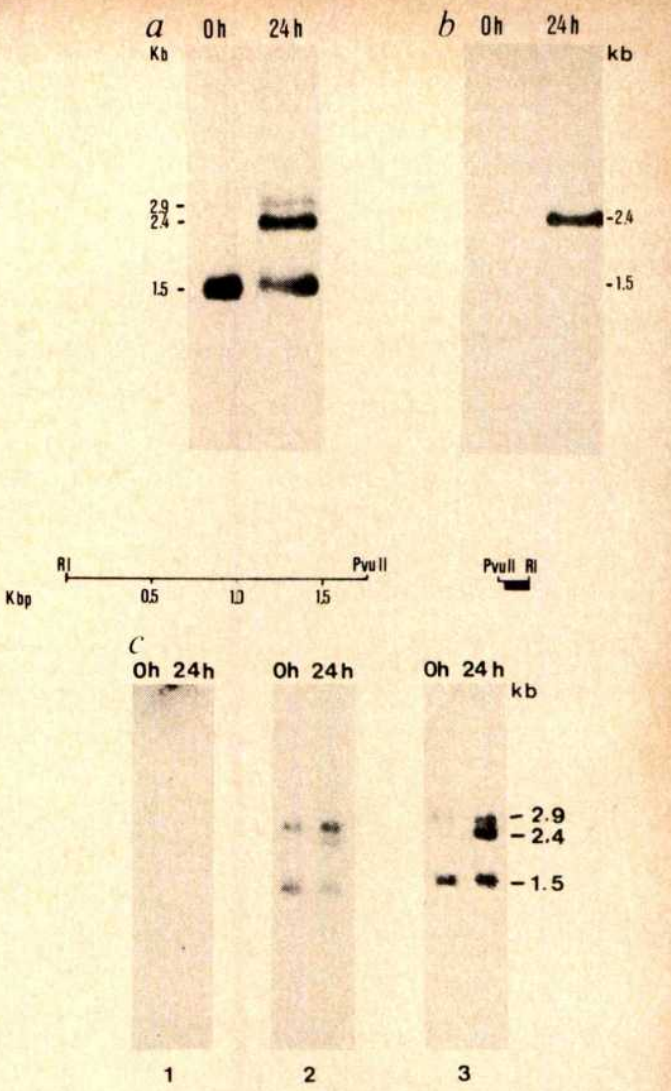


Fig. 6 Hybridization of the flanking (*a*) and homoeo box (*b*) sequences contained in m6-12 DNA and (*c*) single-stranded M13 m6-12 DNA probes to stem-cell (0 h) and differentiated F9 (24 h) cellular RNA. The structures of the probes are shown at the bottom of the figure (solid box represents homoeo box sequences).

Methods. Poly(A^+) RNAs from F9 stem cells and those treated with retinoic acid and dibutyryl cAMP for 24 h were obtained as described in Fig. 5 legend. Samples (5 μg) were separated by electrophoresis in formaldehyde–agarose gels, blotted onto Gene Screen Plus and hybridized to either the nick-translated 1.7-kb *Eco*RI/*Pvu*II fragment (*a*), the 206-bp *Pvu*II/*Eco*RI fragment (*b*) from m6-12 or to the noncoding (1) or coding (2) strands of M13mp10 or mp11 recombinants (*c*) containing the 1.3-kb *Sac*I/*Eco*RI insert from m6-12. As a control, nick-translated pm6-12 (3) was also hybridized to F9 stem-cell (0 h) and differentiated F9 cell (24 h) RNA (*c*). The M13 single-stranded DNA probes were labelled by incorporation of ^{32}P-dCTP using primer extension, denatured in 0.1 M NaOH and electrophoresed in alkali agarose gels. Labelled single strands were recovered by electroelution.

*Pvu*II/*Eco*RI fragment. Because the larger *Pvu*II/*Eco*RI fragment containing the remaining m6-12 sequences hybridizes with all of the transcripts previously seen following differentiation, the 3.2-, 2.9-, 2.4- and 1.5-kb mRNAs presumably all contain these flanking sequences. It is likely, however, that although these mRNAs are transcribed from the strand of DNA that encodes the homoeo box protein open reading frame and map closely on the mouse genome, only the 2.4-kb mRNA contains homoeo box sequences. All of the transcripts that hybridize to the pm6-12 probe were still detected after washing the Northern blots under very stringent conditions (0.1×SSC at 60° C, data not shown).

In attempts to determine whether the induced 2.4-kb mRNA is specific for the production of parietal endoderm, we have performed experiments using several cell types: F9 cells differentiated by treatment with retinoic acid and aggregation into visceral endoderm, P19 cells differentiated into myoblasts[38,39] and mouse embryos isolated at different stages of development. Experiments with retinoic acid-treated F9 cells (visceral endoderm) showed a 2.4-kb mRNA on differentiation similar to that present in parietal endoderm. P19 cells differentiated into myoblasts, as well as mouse embryonic and extra-embryonic tissues, also express mRNAs that hybridize to m6-12 sequences.

We intend to use the different EC cells as model systems to study the role of various homoeotic genes in the differentiation of these cells.

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1. Kleinsmith, L. J. & Pierce, G. B. Jr *Cancer Res.* **24**, 1544-1551 (1964).
2. Martin, G. R. *Science* **209**, 768-776 (1980).
3. Bernstine, E. G., Hooper, M. L., Grandchamp, S. & Ephrussi, B. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3899-3903 (1973).
4. Solter, D. & Knowles, B. B. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5565-5569 (1978).
5. Artzt, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 2988-2992 (1973).
6. Stern, P. L. *et al. Cell* **14**, 775-783 (1978).
7. Paulin, D., Jakob, H., Jacob, F., Weber, K. & Osborn, M. *Differentiation* **22**, 90-99 (1982).
8. Sherman, M. I. & Miller, R. A. *Devl Biol.* **63**, 27-34 (1978).
9. Strickland, S. & Mahdavi, V. *Cell* **15**, 393-403 (1978).
10. Adamson, E. D., Gaunt, S. J. & Graham, C. F. *Cell* **17**, 469-476 (1979).
11. Hogan, B. L. M., Taylor, A. & Adamson, E. *Nature* **291**, 235-237 (1981).
12. Müller, R. & Wagner, E. F. *Nature* **311**, 438-442 (1984).
13. Kahan, B. & Adamson, E. D. *Cold Spring Harb. Conf. Cell Prolif.* **10**, 131-141 (1983).
14. Boller, K. & Kemler, R. *Cold Spring Harb. Conf. Cell Prolif.* **10**, 39-49 (1983).
15. Strickland, S., Smith, K. K. & Marotti, K. R. *Cell* **21**, 347-355 (1980).
16. Levine, R. A., La Rosa, G. J. & Gudas, L. J. *Molec. cell. Biol.* **4**, 2142-2150 (1984).
17. Stacey, A. J. & Evans, M. J. *EMBO J.* **3**, 2279-2285 (1984).
18. McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A. & Gehring, W. J. *Nature* **308**, 428-433 (1984).
19. McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. & Gehring, W. J. *Cell* **37**, 403-408 (1984).
20. Laughon, A. & Scott, M. P. *Nature* **310**, 25-31 (1984).
21. Scott, M. P. *et al. Cell* **35**, 763-776 (1983).
22. Levine, M., Rubin, G. M. & Tjian, R. *Cell* **38**, 667-673 (1984).
23. McGinnis, W., Hart, C. P., Gehring, W. J. & Ruddle, F. H. *Cell* **38**, 675-680 (1984).
24. Carrasco, A. E., McGinnis, W., Gehring, W. J. & De Robertis, E. M. *Cell* **37**, 409-414 (1984).
25. Müller, M. M., Carrasco, A. E. & De Robertis, E. M. *Cell* **39**, 157-162 (1984).
26. Frischauf, A.-M., Lehrach, H., Poustka, A. & Murray, N. *J. molec. Biol.* **170**, 827-842 (1983).
27. Garber, R. L., Kuroiwa, A. & Gehring, W. J. *EMBO J.* **2**, 2027-2036 (1983).
28. Kuroiwa, A., Hafen, E. & Gehring, W. J. *Cell* **37**, 825-831 (1984).
29. Southern, E. *J. molec. Biol.* **98**, 503-517 (1975).
30. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
31. Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560-564 (1977).
32. McBurney, M. W. & Rogers, B. J. *Devl Biol.* **89**, 503-508 (1982).
33. Evans, M. J. & Kaufman, M. H. *Nature* **292**, 154-156 (1981).
34. Gluecksohn-Waelsch, S. *Cold Spring. Harb. Conf. Cell Prolif.* **10**, 3-13 (1983).
35. Walker, J. M., Gooderham, K., Hastings, J. R. B., Mayes, E. & Johns, E. W. *FEBS Lett.* **122**, 264-270 (1980).
36. Geisler, N., Fischer, S., Vandekerckhove, J., Plessmann, U. & Weber, K. *EMBO J.* **3**, 2701-2706 (1984).
37. Adamson, E. D. & Hogan, B. L. M. *Differentiation* **27**, 152-157 (1984).
38. Edwards, M. K. S., Harris, J. F. & McBurney, M. W. *Molec. cell. Biol.* **3**, 2280-2286 (1983).
39. Dony, C., Kessel, M. & Gruss. P. *Cell Differentiation* (in the press).
40. Umene, K. & Enquist, L. W. *Gene* **13**, 251-268 (1981).
41. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294-5299 (1979).

LETTERS TO NATURE

Are galaxies more strongly correlated than clusters?

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One of the most powerful tools used in attempts to understand the structure of the Universe is the correlation function $\xi(r)$, the excess probability over random that there are two objects separated by a distance r. In particular, distributions of galaxies and of clusters of galaxies have been investigated using this parameter. Here we show that if the amplitudes of the cluster–cluster correlation function is made dimensionless, systematic changes with cluster richness vanish, implying scale invariance in the clustering process. The dimensionless galaxy–galaxy correlation seems stronger, implying gravitational enhancement on smaller scales.

It has been established[1] that for distances out to 10 Mpc, the galaxy distribution has a power-law correlation of the form $\xi_{gg}(r) = \alpha_{gg}(hr)^{-1.8}$, where h is the Hubble constant in units of 100 km s^{-1} Mpc^{-1}. Recently , the spatial correlation function of Abell clusters has been determined directly[2,3], following earlier work on the angular correlations[4]. It was noted that rich ($R \geq 1$) clusters of galaxies correlate with the same power law as galaxies, but with a significantly greater amplitude, $\alpha_{cc} = 18\alpha_{gg}$, which is even higher for richer ($R \geq 2$) clusters. Clusters poorer than most Abell clusters were identified in the Lick catalogue by a numerical algorithm[5]. The correlations had the same slope and the amplitude was between that of galaxies and that of the Abell clusters, in agreement with the richness trend (see Table 1).

An attempt to explain the behaviour of α_{cc} has been made by Kaiser[6] applying the statistics of rare events. If the density perturbations are described by a random gaussian field and the regions where clusters form correspond to densities higher than a certain threshold, the correlation function of the points above the 'clipping level' is a constant factor times the correlation function of all the points. By filtering out from the initial power spectrum the scales smaller than clusters and choosing the appropriate threshold, it was possible to match both the number density and enhanced correlations of the Abell clusters. To explain the factor of 20 in α_{cc} the clusters have to be $\geq 3\sigma$ points. In this picture $\xi_{cc}(r)$ will have the same functional form as the galaxy autocorrelations. The model may have some difficulties here, because ξ_{gg} seems to be negative at 20-Mpc radii, but ξ_{cc} is positive out to at least 100 Mpc.

The r.m.s. peculiar velocities and the correlation amplitude of the mass are related; both originate from the power spectrum of perturbations. The clusters cannot represent the true mass autocorrelations in the universe, because then we would expect to see enormous (a few thousand kilometres per second) peculiar velocities for the clusters, which does not seem to be the case.

Whenever similar systems of different scales are compared, it is better to use dimensionless quantities. The correlation amplitude α_{cc} is dimensional; it is $\xi_{cc}(r)$ at a distance $hr = 1$ Mpc. It can be made dimensionless by using an intrinsic unit of length for each sample of different richness. The natural length that appears in these point catalogues is the mean separation L, determined by the density $n = L^{-3}$. This mean separation is indeed intrinsic because there is a unique one-to-one correspondence between richness and the value of L, which is because the richer samples are always subsets of the poorer catalogue. Therefore, labelling the different richness selections by L, the density of each sample is one, and the value of the correlation function at L (expressing distance in units of L) is also dimensionless: $\beta(L) = \xi(L) = \alpha(L)L^{-1.8}$.

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Table 1 The dimensional correlation amplitude $\alpha(L)$, compared with the dimensionless $\beta(L)=\alpha(L)L^{-1.8}$ for groups and clusters of increasing richness

Sample	$\alpha(L)$	n	L	$\beta(L)$
Galaxies	20	8.0×10^{-3}	5	1.10
Schectman	180	3.6×10^{-5}	30	0.39
BS $R\geq1$	360	6.0×10^{-6}	55	0.27
BS $R\geq2$	1,080	2.0×10^{-6}	80	0.41

n, The comoving spatial density in Mpc^{-3}; $L=n^{-1/3}$, the mean separation in Mpc. The spatial density of galaxies is given in ref. 15.

The constant slope in the power law behaviour of $\xi(r)$ indicates scale invariance of the process responsible for galaxy formation. In the case of galaxies, the typical distances are a few Mpc, whereas for clusters the same law holds to distances >50 Mpc. If galaxy formation were completely scale invariant, one would expect that all dimensionless correlation amplitudes would be equal.

Figure 1 is a plot of the dimensionless amplitude $\beta(L)$ for all the samples, including the different richness classes of Bahcall and Soneira[2]. Note that $\beta(L)$ for galaxies is 1.1, a factor of three higher than for the cluster samples, which are all consistent with 0.35. As far as clusters are concerned, the scale invariance seems to hold. The correlation amplitudes of the cluster catalogues are fairly well known, but their densities are less certain. Because L and thus $\beta(L)$ depend on the density, we plotted conservative error limits on Fig. 1 corresponding to densities 1.5 times above and below the quoted value. Thus compared, galaxies are more strongly correlated than clusters, the opposite conclusion to that obtained by comparing $\alpha(L)$.

The relative constancy of $\beta(L)$ can be understood in general terms as a signature of scale invariance. If there is a nonlinear process (besides gravity) modulating galaxy formation and this process is scale invariant, the created pattern will have a single power law correlation function[7], $\xi(r)=\beta(r/L)^{D-3}$, the slope of which would be related to the geometry of the pattern, namely to its fractional (or 'fractal') dimension D. This $\xi(r)$ will have the correct scaling property. Thus, to explain the observed slope, a fractal dimension of $D=1.2$ is required, but we do not know yet what physical process can generate the correct D. (We discuss several possibilities below.)

Small-scale gravitational clustering may break the scale invariance and increase the dimensionless correlation amplitude for galaxies. In one extreme case, we can approximate this process by assuming that the pattern imposed on the galaxy distribution is uncorrelated with the gravitational motion. Then the observed correlations are a superposition of the gravitational and fractal correlations, with $\xi_{frac}(r)=0.35(r/L)^{-1.8}$, which is scale invariant, and $\xi_{grav}(r)$ a flat function of r out to ~10 Mpc, independent of L. ξ_{grav} Is the Fourier transform of the power spectrum: $1+\xi_{obs}(r)=[1+\xi_{grav}(r)][1+\xi_{frac}(r)]$. If $\xi_{grav}(0)\approx3$, then we approximately reproduce the observed factor of three in $\beta(L)$.

In the other extreme the process is the nonlinear 'clipping' of the gravitationally induced overdensities[6], in which case the 'fractal' and the gravitational perturbations are fully correlated. Kaiser has also found a limited scale invariance for that particular model. If the selection is simply the result of a threshold in the initial perturbations, which are otherwise scale invariant, the resulting regions, the so-called 'excursion sets', will have a scale-invariant geometry and will be fractals[8]. If the universe is dominated by 'cold' dark matter, the fluctuation spectrum has an asymptotic form of k^{-3}, where k is the wavenumber. The larger galaxy correlations in this case can be attributed to effects of nonlinear gravitational clustering on small scales. From comparisons of the low r.m.s. galaxy velocities and the high correlation function of galaxies, it seems that such a 'bias' is useful[9].

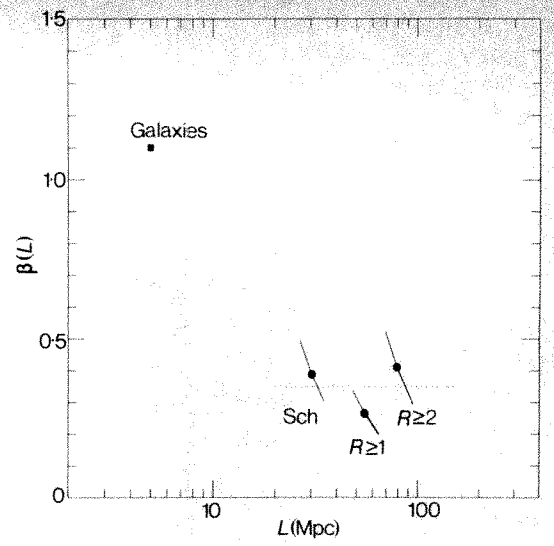


Fig. 1 The dimensionless correlation amplitude $\beta(L)$ is shown as a function of the mean separation L. Note that all three cluster catalogues are consistent with 0.35 (dashed line), whereas the galaxy amplitude is 1.1. The error-bars in the cluster data represent a factor of 1.5 up/down uncertainty in the density.

All these mechanisms have the advantage of increasing the galaxy correlations while leaving the velocities intact.

Schulman and Seiden[10] have already suggested similarities between condensed matter physics and galaxy formation. Their conclusion, from simple analogies with short range interaction spin systems, was that only fractals with $D\sim2$ can be obtained.

Gas dynamical processes are bound to occur at some level during galaxy formation. The formation and explosion of first generation Pop III stars[11,12] could have major consequences for the entire galaxy formation scenario. Although the energies available in each explosion are insufficiently large, percolation may occur, creating random structures of 'infinite' length that can produce the required behaviour.

Another possibility is relativistic strings[13,14], which have attracted much attention recently. If present, they may create patterns with a fractal dimension close to unity.

In conclusion, we propose that galaxy and cluster correlation functions be compared in dimensionless units. The dynamic range of correlation amplitudes has been reduced from 50 to 3. All cluster correlations are consistent with the same scale-invariant amplitude. That in those units galaxies are more strongly clustered may be because of small-scale gravitational clustering, whereas the slope and the dimensionless amplitude may reflect a scale invariant process responsible for creating luminous galaxies in certain regions of the universe.

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1. Groth, E. J. & Peebles, P. J. E. *Astrophys. J.* **217**, 385-405 (1977).
2. Bahcall, N. A. & Soneira, R. A. *Astrophys. J.* **270**, 20-38 (1983).
3. Klypin, A. A. & Kopylov, A. I. *Sov. Astr. Lett.* **9**, 41-46 (1983).
4. Hauser, M. G. & Peebles, P. J. E. *Astrophys. J.* **185**, 757-786 (1973).
5. Schectman, S. A. *Astrophys. J.* (in the press).
6. Kaiser, N. *Astrophys. J.* **284**, L9-L12 (1984).
7. Mandelbrot, B. B. *Fractals* (Freeman, San Francisco, 1977).
8. Adler, R. J. *The Geometry of Random Fields* (Wiley, New York, 1981).
9. White, S. D. M. *Proc. Inner Space/Outer Space* (University of Chicago Press, 1985).
10. Schulman, L. S. & Seiden, P. E. *IBM Rep.* RC-10369 (1984).
11. Carr, B. J., Bond, J. R. & Arnett, D. *Astrophys. J.* **277**, 445-469 (1984).
12. Carr, B. J. & Ikeuchi, S. *Kyoto Preprint* RIFP-550 (1984).
13. Turok, N. & Albrecht, A. *ITP Santa Barbara Preprint* (1984).
14. Turock, N. & Schramm, D. N. *Nature* **312**, 598-599 (1984).
15. Davis, M. & Huchra, J. *Astrophys. J.* **254**, 437-454 (1982).

A new non-thermal galactic radio source with a possible binary system

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Helfand and Becker[1] recently discussed the unusual filamentary appearance of the galactic radio sources G357.7–0.1 and G5.3–1.0, both of which had originally been classified as supernova remnants (SNR$_s$)[2,3], and proposed that these sources belong to a new class of non-thermal radio sources that originate in accreting binary systems containing a neutron star or a black hole. Becker and Helfand[4] have pointed out that other galactic sources may be identified as members of this class; we report here our claim that a new non-thermal galactic object (G18.95−1.1), detected in the recently published 2.695-GHz galactic plane survey[5], is a possible candidate. That the integrated flux density spectral index $\alpha = -0.4$ ($S_\nu \sim \nu^\alpha$) and the polarization is ≈2.5% at 4.75 GHz, proves the non-thermal nature of the new source; morphologically, a classification of this object as a SNR seems impossible. G18.95−1.1 consists of various arcs all pointing towards a central radio peak; we suggest that the object is a binary system containing a compact component, located at or close to this radio peak, accelerating electrons to relativistic energies—observed in the arcs by the synchrotron emission of the accelerating electrons.

The source G18.95−1.1 has subsequently been observed at 1.42 GHz and 4.75 GHz with the Effelsberg 100-m telescope and at 10 GHz with the Nobeyama 45-m dish. The 4.75-GHz map is shown in Fig. 1 and the spectrum is plotted in Fig. 2. Both the spectral index and the polarization reveal the non-thermal nature of G18.95−1.1.

How do we classify this object? The peak radio intensity is close to the centre of the object and shows a bar-like structure with peaks near each end of the bar (Fig. 1). Even the highest available angular resolution does not allow separation of any compact source from the diffuse emission. The grey-scale radiograph in Fig. 3 allows the determination of the source structure in much greater detail. From this presentation it is apparent that G18.95−1.1 consists of various arcs pointing towards the radio peak slightly off-centre to the north-east. Clearly, the morphology of G18.95−1.1 defies any classification as a SNR and also contradicts the general appearance of radio galaxies. Its arcs may be of similar origin to the filaments in G357.7−0.1 discussed by Helfand and Becker[1]. We therefore suggest a binary system containing a compact component as the source of G18.95−1.1. This possible centre of activity should be expected to be located in the central bar, perhaps at or close to the peak emission north-east of the centre. If the central location of this source is not the result of projectional effects, the velocity of the binary system relative to the filamentary structure is low, or the source is much younger than G357.7−0.1 and G5.3−1.0.

The estimation of source parameters (for example, age, distance) is hindered by the insufficient data available. We suggest that deep optical observations (no corresponding emission could be found in the Palomar plates) and a search for X-ray emission be made. At radio wavelengths, more sensitive polarization

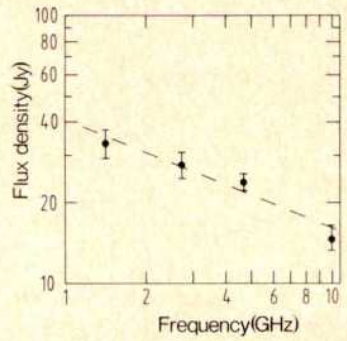


Fig. 2 The radio spectrum of G18.95−1.1. The integrated flux densities are: $S(1.42\ \text{GHz}) = 32.9$ Jy, $S(2.695\ \text{GHz}) = 27.4$ Jy, $S(4.75\ \text{GHz}) = 23.8$ Jy and $S(10\ \text{GHz}) = 14.6$ Jy; the typical error is 10%. The mean flux density spectral index $\alpha = -0.4$. There may be a steepening of the spectrum from $\alpha = -0.4$, in the inner part near the radio peak, to $\alpha = -0.5$ at the outer arcs, but the accuracy of the spectral index is 0.2. The errors are mainly attributable to the uncertainty in the estimation of the base levels.

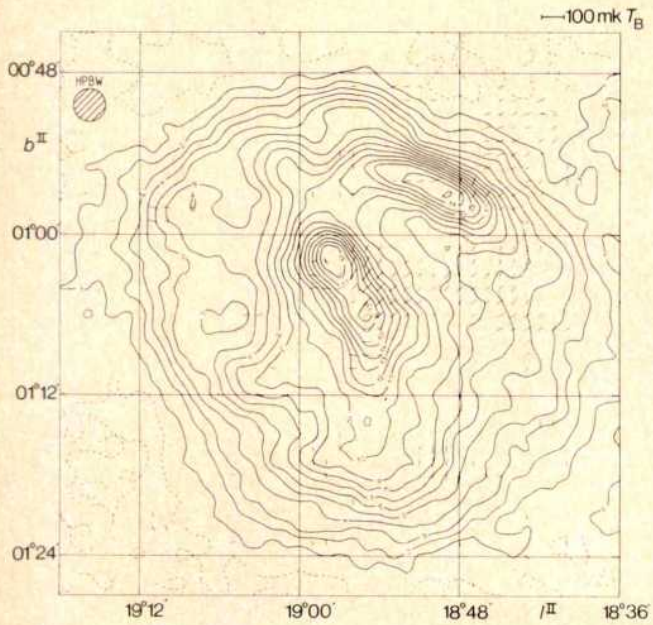


Fig. 1 4.75-GHz map of G18.95−1.1. Contour steps are 50 mK brightness temperature T_B. The angular resolution is 2.4 arc min. Superposed are the linear polarization intensity E-vectors. The integrated polarization percentage is 2.5%.



Fig. 3 The grey-scale map of G18.95−1.1.

measurements together with improved angular resolution (possibly using the Very Large Array) and a search for a pulsar will certainly help to understand the nature of G18.95−1.1. If our assumption of a binary system as the source of G18.95−1.1 is confirmed, this object will provide important information on the interaction of binary systems with the surrounding interstellar medium.

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1 Helfand, D J & Becker, R H *Nature* **313**, 118–119 (1985).
2 Milne, D E *Aust J Phys* **32**, 83–92 (1979)
3 Green, D A *Mon Not R astr Soc* **209**, 449–478 (1984)
4 Becker, R H & Helfand, D J *Nature* **313**, 115–118 (1985)
5 Reich, W., Furst, E., Steffen, P., Reif, K. fi Haslam, C G T *Astr Astrophys Suppl* **58**, 197–248 (1984)

A higher limit to the mean galaxy mass allowed by gravitational lens image distortion

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Tyson *et al.*[1], in a recent discussion of the gravitational lens distortion, by foreground galaxies, of the images of distant ('background') galaxies, claim that their non-detection of this effect implies a low mean mass for the foreground galaxies. The effect they sought is a change in the coherent ellipticity, that is, the ratio of the angular diameters orthogonal and parallel to the separation vector to the foreground galaxy. Using samples of galaxies in different ranges of magnitude to represent the 'foreground' ($19 < J < 21.5$) and 'background' ($22.5 < J < 23.5$) galaxies, they found no significant effect and therefore deduced a 2σ upper limit to the mean mass of the foreground galaxies of only $2.8\times10^{11}\ H^{-1}\ M_\odot$ out to a radius of $80\ H^{-1}$ kpc (where H is Hubble's constant in units of 100 km s^{-1} Mpc^{-1}). The corresponding mass-to-light ratio and the known luminosity density of the Universe then requires that the mass density in the form of galaxies is $\Omega_{gal} < 0.03$, which is in marked contrast to the values $\Omega_{gal} \sim 0.2$ obtained by various dynamical arguments, namely, the rotation curves of individual galaxies, the virial equation applied to groups and clusters and the cosmic virial theorem[2–5]. I show here that, by including the effect of galaxy clustering, the limit on Ω_{gal} is indeed raised to $\Omega_{gal} \leqslant 0.2$, in accordance with the dynamical determinations.

Tyson *et al.*[1] noted that for the two magnitude ranges they used, there should be little overlap in the distances of their two sets of galaxies, and substantiated this by Monte Carlo experiments using a Schechter[6] luminosity function. They claimed therefore that almost all observed pairs should be a genuine background–foreground pair (the lensing effect is insignificant if the two galaxies are close together). However, this step is invalid if galaxy clustering is taken into account, because for any observed pair, what we really require is the conditional probability of finding a 'background' (that is, faint) galaxy at a particular distance given the presence of the foreground galaxy, since this becomes significantly confused by nearby clustered low-luminosity galaxies. It is easy to see that this has a substantial effect by considering the standard form for the galaxy autocorrelation function[7], $\xi(s) = (s/r_0)^{-\gamma}$, with index $\gamma = 1.8$ and scale length $r_0 \approx 5\ H^{-1}$ Mpc. Even at the maximum pair separation used by Tyson *et al.*, corresponding to $220\ H^{-1}$ kpc at the mean distance $D = 720\ H^{-1}$ Mpc of the foreground galaxies, the clustering increases the number of expected neighbours by factors of several hundred relative to that for a random distribution.

More exactly, we can integrate the correlation function to show that the number of galaxies per unit solid angle at separation θ from a given foreground galaxy, distance D from the observer, will be[8]

$$N' = N + D^2\Phi(M)G(\gamma)r_0^\gamma(\theta D)^{1-\gamma}$$

where N is the mean surface density, Φ is the integral luminosity function and $G = 3.678$ for $\gamma = 1.8$. Now, from Tyson *et al.*, we find the mean background density to be 5,670 galaxies per deg^2 or $N = 1.86\times10^7$ rad^{-2}. Also, with the previously specified values of D, G, r_0 and γ and taking[9] $\Phi \approx 0.05\ H^3$ Mpc^{-3} (in this context Φ is the number density of galaxies in the magnitude range $-16.5 \geqslant M_B - 5\log H \geqslant -17.5$ corresponding to $22.5 < J < 23.5$ at $D = 720\ H^{-1}$ Mpc), the second term on the right-hand side has the value $1.7\times10^8\ \theta_s^{-0.8}$, where θ_s is the separation in arc seconds.

In other words, the fraction of genuine background–foreground pairs that are able to show a measurable lensing effect is only $1/(1+9\ \theta_s^{-0.8})$ so that the mean distortion will be diluted by a factor ranging from 4.0 at $\theta = 4$ arc s to 1.5 at $\theta = 40$ arc s. (Note that the ratio will be the same even if there is a bias against detecting close pairs, because of the presence of the bright foreground galaxy, thus reducing the apparent clustering.) As the effect sought is greatest at small separations, this obviously greatly limits the power of the test. When the dilution factor is included, even the most massive model considered by Tyson *et al.* (outer radius $190\ H^{-1}$ kpc, equivalent circular velocity 300 km s^{-1}, that is, mass $\approx 2.1\ H^{-1}\times10^{12}\ M_\odot$) is within 2.5σ of the observed points for all except one of the bins in θ. It may therefore be prudent, at present, to take a conservative upper limit of $\sim 2\times10^{12}\ H^{-1}\ M_\odot$ for the mass of a mean foreground galaxy, which raises the limit on the density parameter to $\Omega_{gal} \leqslant 0.2$. We note also that if the clustered galaxies are preferentially aligned[10], then the situation is even worse than we have assumed and almost any density parameter will be allowed, because this effect will work in opposition to the lens effect that causes an orthogonal elongation.

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1 Tyson, J A, Valdes, F, Jarvis, J F & Mills, A P *Astrophys J* **281**, L59–L62 (1984)
2 Rubin, V C, Ford, W. K & Thonnard, N *Astrophys J* **225**, L107–L112 (1978)
3 Faber, S M & Gallagher, J S *A Rev Astr Astrophys* **17**, 135–187 (1979)
4 Peebles, P J E in *Physical Cosmology* (eds Balian, R, Audouze, J & Schramm, D N) (North-Holland, Amsterdam, 1980)
5 Bean, A J, Efstathiou, G., Ellis, R S, Peterson, B A & Shanks, T *Mon Not. R. astr Soc.* **205**, 605–624 (1983)
6 Schechter, P *Astrophys, J* **203**, 297–306 (1976)
7 Peebles, P J E *The Large Scale Structure of the Universe* (Princeton University Press, 1980)
8 Phillipps, S *Mon Not R astr Soc* **212**, 657–661 (1985)
9. Felton, J E, *Astr J* **82**, 861–878 (1977)
10 Djorgovski, S *Astrophys J* **274**, L7–L12 (1983)

Synthesis of ethylene and ethane by partial oxidation of methane over lithium-doped magnesium oxide

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The partial oxidation of methane into more useful chemicals such as methanol, ethylene and benzene has been investigated extensively, although yields for these products have been poor[1–4]. Moreover, in several of these processes the required oxidant is N_2O rather than O_2. Recent work[5] in our laboratory has demon-

Table 1 Results for the partial oxidation of methane at 720 °C over Li/MgO

	Partial pressure (torr)								
	Reactants		Products				Conversion†	C_2 selectivity	C_2 yield
Run no	CH_4	O_2	CO_2	CO	C_2H_4	C_2H_6	%	%	%
1	59	29	11.2	0.0	3.5	2.2	37.8	50.3	19.0
2	58	36	13.7	0.2	3.7	2.1	42.8	45.4	19.4
3	89	29	11.1	0.0	4.6	3.1	29.1	58.1	16.9
4	218	117	51.2	1.5	15.4	7.4	37.5	46.5	17.4

In earlier runs (not listed), Li/MgO was used at 770 °C. Values for C_2 selectivity and C_2 yield were based on the amount of methane that had reacted. C_2 yield is defined as the product of conversion and C_2 selectivity.

strated that lithium-doped magnesium oxide (Li/MgO) in the presence of O_2 has high activity for abstracting H from CH_4 to form $\cdot CH_3$ radicals. This suggests that C_2H_6 and C_2H_4 (C_2 compounds) are produced by a coupling between two gaseous $\cdot CH_3$ radicals formed on this catalyst. We report here our success in converting CH_4 to C_2 compounds in high yields in conventional catalytic conditions.

Powder catalysts of Li/MgO, containing 3 and 7 wt% Li, were prepared from Li_2CO_3 and MgO[5] and then activated at 465 °C in an oxygen flow. The catalytic experiments were performed at 1 atm in a conventional flow reactor of the type described previously[4]. A reacting gas mixture of CH_4, O_2 and He (a diluent) was introduced at a flow rate of 0.83 ml s^{-1}. All of the gases were analysed by gas chromatography except HCHO, which was analysed by a titration technique[4].

High yields for the C_2 compounds were attained when the ratio of O_2 to CH_4 in a reactant mixture was ~0.5. The results, which were obtained at 720 °C over 4 g of 7% Li/MgO, are summarized in Table 1. Besides C_2H_4, C_2H_6, CO_2 and CO, CH_3OH and HCHO also were produced, but in only trace amounts. By comparison, the conversion of CH_4 was 0.2% at 720 °C in the same experimental conditions except for the absence of the catalyst. Pure MgO catalyst (1 g), prepared by the same method as Li/MgO but without Li_2CO_3, produced the C_2 compounds with a low selectivity of 6% at a conversion level of 17% at 700 °C.

A yield of 19% for the C_2 compounds was obtained with 50.3% selectivity at 37.8% conversion (run 1), which is considerably better than results reported in the literature for other metal oxide catalysts[1,3]. Almost equal amounts of CH_4 and O_2 reacted with each other, and most of the O_2 was consumed during the reaction in these experimental conditions. By feeding a reactant gas mixture containing O_2 in a higher ratio than that of run 1, we could attain a higher conversion of CH_4, but the selectivity for the C_2 compounds decreased (run 2). In contrast, a reactant mixture of the lower O_2/CH_4 ratio gave a better C_2 selectivity but a poorer conversion (run 3). Run 4 shows that a good yield of C_2 was obtained even at a higher partial pressure of the reactants if the O_2/CH_4 ratio was kept at ~0.5. Although C_2H_4 is the main C_2 compound over Li/MgO, C_2H_6 is also produced in considerable amounts. From the practical viewpoint, it is desirable that C_2H_6 is converted into C_2H_4 and the technology for this process is available.

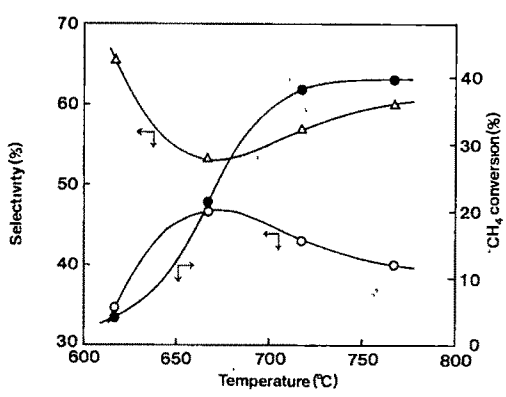


Fig. 1 Effect of the reaction temperature on the partial oxidation of methane. A reactant mixture containing 291 torr of CH_4 and 155 torr of O_2 was fed over 4 g of 3 wt% Li/MgO. Selectivities ○, C_2 compounds; △, CO plus CO_2, ●, per cent conversion.

Figure 1 shows effects of reaction temperature on the CH_4 conversion and the selectivities, which were measured over 4 g of 3% Li/MgO. This reaction system is unusual in that the selectivity for C_2 compounds increases as the CH_4 conversion increases up to a temperature of 670 °C. In most heterogeneous oxidation catalysis, the selective oxidation decreases with increasing conversion. The phenomenon in Fig. 1 may be understood in terms of two primary reaction pathways for the $\cdot CH_3$ radicals; they may either couple to form C_2H_6 (ref. 6) or they may react with the oxide surface to form surface methoxide ions that ultimately result in the production of CO and CO_2 (ref. 7). The former channel is second order with respect to $[\cdot CH_3]$ and is thus favoured at high temperatures where more $\cdot CH_3$ radicals are produced. One cannot, of course, rule out the possibility that part of the CO and CO_2 is formed by a homogeneous reaction between $\cdot CH_3$ and O_2. The C_2H_6 may be converted to C_2H_4, also through alkyl radicals, but further oxidation to CO and CO_2 seems to be retarded below 670 °C in the presence of CH_4.

The $\cdot CH_3$ radicals are believed to be formed at (Li^+O^-) centres on MgO (ref. 5) which have been observed in Li-doped MgO single crystals[8] and more recently in the sample used in these experiments[5]. Gas phase and surface studies have shown that O^- ions efficiently abstract H· from CH_4 (refs 7, 9). The Li/MgO catalyst does not contain ions having variable oxidation states or transition metals; thus, it is a new type of catalyst for the activation of methane.

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1 Keller, G E, & Bhasin, M M *J Catal.* **73**, 9-19 (1982)
2 Atroshchenko, V I, Shchedrinskaya, Z M & Gavrya, N A *Zh prikl Khim, Mosk.* **38**, 643-649 (1965)
3 Hinsen, W & Baerns, M *Chemikerzeitung* **107**, 223-226 (1983)
4 Liu, H-F, Liu, R-S, Liew, K Y, Johnson, R E & Lunsford, J H *J Am chem Soc.* **106**, 4117-4121 (1984)
5 Driscoll, D J, Martir, W, Wang, J-X & Lunsford, J H *J Am chem Soc* **107**, 58-63 (1985)
6 Pollard, R T. *Compreh chem Kinet* **17**, 249-367 (1977)
7 Aika, K & Lunsford, J H *J phys Chem* **81**, 1393-1398 (1977)
8 Chen, Y, Tohver, H T, Narayan, J & Abraham, M M *Phys Rev* **B16**, 5535-5542 (1977)
9 Bohme, D K & Fehsenfeld, F C *Can J Chem* **47**, 2717-2719 (1969)

Highly conducting acetylene–CO copolymers and limitations of the soliton model

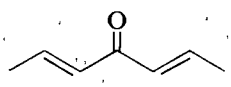
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There is much scientific and technological interest in conductive polymers, of which polyacetylene is the simplest and best example, and in the mechanism of carrier transport. *Trans*-$[CH]_x$ with C_{2h} symmetry is said[1–4] to have an electronically degenerate ground state with very slight alternation of backbone bond lengths, that is, a high degree of conjugation. Radicals or radical ions in the chain are referred to as soliton[5,6] and polaron[7] excitations with moving domains, but these rigorous concepts are quite restrictive and exclusive. For instance, moving-domain excitations cannot exist in *cis*-$[CH]_x$ because much energy is required for the interconversion of *cis*-transoid and *trans*-cisoid structures. However, many physical and spectroscopic properties of undoped and doped *trans*-$[CH]_x$ have been explained by these models[8]. An intersoliton electron-hopping model had been proposed for carrier transport in lightly doped *trans*-$[CH]_x$[9]. Our purpose here is to test the general validity of the soliton/polaron models. Our present results cast strong doubts on the relevance of these theoretical models to carrier transport in conductive polymers, thereby allowing a search for possible new conductive polymers that is free of the constraints imposed by these models.

Copolymerizations of acetylene and CO were initiated by the $Ti(OBu)_4/4AlEt_3$ catalyst at −78 and 25 °C for 18–24 h at monomer feed ratios (C_2H_2/CO) of 4/1, 2/1 and 1/1 to give copolymers of composition 95/5, 90/10 and 87/13, respectively. The number-average relative molecular masses are ~3,900 ± 300 and 970 for copolymers obtained at −78 and 25 °C, respectively, as determined by quenching with tritiated methanol[10–12].

We have the following evidence that copolymers were obtained: (1) Elemental analysis (Table 1). (2) Radioassay of ^{14}CO-containing copolymers. (3) Magic angle spinning ^{13}C-nuclear magnetic resonance (NMR) showed a carbonyl resonance at 178 p.p.m.[13] of the correct intensity. (4) Two carbonyl bands at 1,670 and 1,720 cm^{-1} attributable[14] to the C=O vibrations in



and $-CH=CH=\overset{O}{\overset{\|}{C}}-H$

respectively; the latter was probably formed on quenching of Ti—C(=O)—P with methanol. (5) Copolymers formed directly on electron microscope grids[15] are devoid of the characteristic fibrillar morphology of polyacetylene[16] and are completely amorphous according to electron diffraction[17,18]. (6) The *cis*-content in the pristine copolymer decreases markedly with the increase of CO monomer; probably, the initially formed *cis*-transoid structures[19] are more easily isomerized to the *trans* structure because of the presence of the single bonds in the —C(=O)— units and the absence of crystallinity. (7) Thermogravimetric analysis of copolymers showed a progressively larger weight loss at lower temperatures with increasing amount of CO, with the weight loss approximately equal to the CO in the copolymer. (8) Pyrolysis GC–MS of the copolymers produced almost the same products as that of polyacetylene[20] but with CO and some CO_2 and H_2O. (9) Treatment of the copolymer with protonic acid greatly reduces the intensities of the 178 p.p.m. ^{13}C-NMR resonance and the infrared carbonyl bands as expected for facile protonation of carbonyl groups activated by adjacent π systems. Lastly, (10) the copolymer films have a dull coppery colour on the side facing the reactor wall, but the other side has various shiny green colours that depend on the monomer feed ratio and which are different from those of polyacetylene—indicative of the wider band gap for the copolymer than for polyacetylene.

The key magnetic and transport properties of undoped poly(acetylene-*co*-CO) are summarized in Table 2. For simplicity, we will refer to the samples poly(95acetylene-*co*-5CO), poly(90acetylene-*co*-10CO) and poly(87acetylene-*co*-13CO) as

Table 1 Comonomer feed and copolymer composition

Sample	Mole ratio C_2H_2/CO in feed	Elemental analysis* (mol %) C	H	C_2H_2	C_2H_2(mol %) By radioassay†	By ^{13}C-NMR‡
A	4	89.43	7 85	95	96	95
B	2	86.34	7.82	88	91	90
C	1	84.90	8.34	85	89	ND

* Microanalysis Laboratory, Massachusetts University, Ti and Al analyses were both 0.2–0.3% as in normal polyacetylene.
† Radioassay (NEN) of copolymers obtained with isotopic carbon monoxide.
‡ ND, not determined

Table 2 Properties of undoped acetylene–CO copolymers

Sample	*cis* Content (%)‖	ΔH_{pp}(G), 25 °C	Spin per repeat unit (+10^5)	σ_{RT} ($(\Omega\ cm)^{-1}$)	S_{RT} ($\mu V\ K^{-1}$)
'*cis*'-A*	83	5.5	29	3×10^{-10}	
trans-A†	0	1.1	2.2	4×10^{-5}	
'*cis*'-B‡	58	5.0	29	$1\,4\times10^{-7}$	
trans-B†	0	1.1	8 3	$1\,7\times10^{-6}$	1,760
'*cis*'-C§	37	3.0	4.0	3.5×10^{-5}	1,600
trans-C†	0	1.2	5.0	1.3×10^{-5}	1,640
cis-$[CH]_x$	~85¶	8–10¶	670–2,000¶		
trans-$[CH]_x$	<5¶	0.5–1.0¶	6,700–20,000¶		1,450

* Poly(95acetylene-*co*-5CO); † thermally isomerized from the '*cis*' polymer at 180 °C for 10 min; ‡ poly(90acetylene-*co*-10CO); § poly(87acetylene-*co*-13CO).
‖ By infrared.
¶ Range of values in literature

Table 3 Conductivity of doped acetylene-carbon monoxide copolymers

Sample*	y†(I_3^-)	$\sigma_{RT}((\Omega\,cm)^{-1})$	y (AsF_5)	σ_{RT} ($(\Omega\,cm)^{-1}$)	S_{RT} ($\mu V\,K^{-1}$)
cis-$[CH]_x$	0.08	~100	0.12	~400	~+20
p-**A**‡	0.06	390	0.09	230	+25
p-**B**	0.05	98	0.03	95	+27
p-**C**	0.04	129	0.04	161	+18

* Sample composition as in Table 1. † *y*, Mol. dopant per repeat unit ‡ *p*, Pristine AC-copolymers.

A, B and C, respectively. The pristine AC-copolymers have room temperature (RT) peak-to-peak linewidths ΔH_{pp} for their unpaired-spin EPR that are comparable to, but smaller than, for *cis*-$[CH]_x$; the differential is larger for copolymers with more CO content. Their linewidths increased to 6 G at 134 K, consistent with low diffusivity of the unpaired spins. Thermal isomerization of the AC-copolymers greatly reduces the number of unpaired spins to only 10^{-4}–10^{-3} of the number in *trans*-$[CH]_x$, but the isomers have comparable RT EPR linewidth and motional narrowing. In sample C, there is on the average one —C(=O)— unit separating segments containing about six $+CH=CH+$ units, the length of which corresponds to the estimated domain widths for soliton[21] and polaron[7]. The EPR of *trans*-AC-copolymers shows about the same motional narrowing as in *trans*-$[CH]_x$. This is either because local processes cause line narrowing or because the —C(=O)— unit does not present a significant barrier to moving domain. Furthermore, the spin-lattice and spin-spin relaxation times are within a factor of two for the two sets of polymers—pristine AC-copolymers and *cis*-$[CH]_x$ and isomerized AC-copolymers and *trans*-$[CH]_x$. Finally, the undoped AC-copolymers all have conductivity σ_{RT} and thermopower coefficient S_{RT} comparable to the corresponding undoped $[CH]_x$.

The values of σ_{RT} for iodine-doped pristine AC-copolymers are either comparable to, or greater than, those for iodine-doped *cis*-$[CH]_x$ (Table 3, column 3). The σ_{RT} values of AsF_5-doped AC-copolymers are one-half to one-fourth those of similarly doped *cis*-$[CH]_x$. The S_{RT} values, indicative of the intrinsic conductivity of the material, are almost the same for AsF_5-doped AC-copolymers and *cis*-$[CH]_x$ (Table 3, column 6).

In conclusion, the AC-copolymers do not have the molecular and supermolecular structures required for soliton and/or polaron excitations; yet those magnetic and transport properties heretofore said to be uniquely belonging to *trans*-$[CH]_x$ and attributed to soliton and/or polaron are manifest in the copolymers. The fact that an extremely low unpaired spin concentration in isomerized AC-copolymers occurs without any loss in conductivity, unlike in *trans*-$[CH]_x$, invalidates any transport mechanism invoking the participation of neutral soliton. Of course, the situation is very complicated for the heavily doped polymers and much more research has to be done before elucidating further the conduction mechanism. The significance of this work is that the results obviate the rigid and restrictive present theoretical framework for conducting polymers and open up possibilities for creative designing and synthesis of new polymers. Furthermore, it eliminates the need to force the soliton/polaron concept on poly(pyrrole), poly(*p*-phenylene vinylene), poly(*p*-phenylene), poly(phenylene sulphide), poly(*p*-aniline) and others that do not have electronic ground-state degeneracy. A conductive polymer needs to have only short conjugated segments to facilitate the redox doping and stabilize the resulting charge carriers. A similar conclusion was reached by MacDiarmid *et al.*[22], who had homogeneously introduced sp^3-defect sites of the type —CHD— into polyacetylene and found that the $+(CH=CH)_nCHD+_x$ material has high conductivity with $\sigma \propto n^{3.2}$ dependence on the conjugation length for $n \geq 3$.

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1 Shirakawa, H, & Ikeda, S *Polym J* **2**, 231–244 (1971)
2 Peierls, R E *Quantum Theory of Solids*, 108–110 (Clarendon, Oxford, 1955)
3 Chien, J C W, Karasz, F E & Shimamura, K *Makromol Chem Rapid Commun* **3**, 655–659 (1982)
4 Fincher, C R Jr; Chen, C E, Heeger, A J, MacDiarmid, A G & Hastings, J B *Phys Rev Lett* **48**, 100–104 (1982)
5 Su, W P, Schrieffer, J R & Heeger, A J *Phys Rev Lett* **42**, 1698–1701 (1978)
6 Rice, M J *Phys Lett* **71A**, 152–154 (1979)
7 Campbell, D K & Bishop, A R *Phys Rev B* **24**, 4859–4862 (1981)
8 Chien, J C W *Polyacetylene—Chemistry, Physics and Material Science* (Academic, New York, 1984)
9 Kivelson, S *Phys Rev B* **25**, 3798–3821 (1982)
10 Chien, J C W, Capistran, J D, Karasz, F E, Dickinson, L C & Schen, M A *J polym Sci polym Lett Ed* **21**, 93 (1983)
11 Chien, J C W, Karasz, F E, Schen, M A & Hirsch, J A *Macromolecules* **16**, 1694–1697 (1983)
12 Schen, M A, Karasz, F E & Chien, J C W *J polym Sci polym. Chem Ed* **21**, 2787–2812 (1983)
13 Chien, J C W & Babu, G N *Macromolecules* (in the press)
14 Bellamy, L J *The Infra-red Spectrum of Complex Molecules*, 1–132 (Wiley, New York, 1958)
15 Karasz, F E *et al Nature* **282**, 286–288 (1979)
16 Chien, J C W *et al Nature* **299**, 608–611 (1982)
17 Shimamura, K, Karasz, F E, Hirsch, J A. & Chien, J C W *Makromol Chem Rapid Commun* **2**, 473–480 (1981)
18 Chien, J C W., Karasz, F E & Shimamura, K *Macromolecules* **15**, 1012–1017 (1982)
19 Chien, J C W., Karasz, F E, Wnek, G E, MacDiarmid, A G & Heeger, A J *J polym Sci polym Lett Ed* **18**, 45–52 (1979)
20 Chien, J C W, Uden, P C & Fan, J L *J polym Sci polym Chem. Ed* **20**, 2159–2167 (1982).
21 Weinberger, B R, Ehrenfreund, E, Pron, A, Heeger, A J & MacDiarmid, A. G *J chem Phys* **72**, 4749 (1980)
22 Yaniger, S I, Kletter, M J & MacDiarmid, A G *188th Am. Chem Soc Abstr Polym* **154**, (Philadelphia, 1984)

Crustal structure south of the Iapetus suture beneath northern England

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The crustal structure just south of the Iapetus suture[1] along a line extending from the mid-North Sea high across northern England to the Solway Basin has been investigated as part of the Caledonian Suture Seismic Experiment of 1982. This unique sea-to-land wide-angle explosion seismology project has produced data of unprecedented quantity and quality. The main innovation was the use of both closely spaced shots (mainly at sea) and closely spaced stations (mainly on land), yielding a data set which can eventually be arranged in a variety of new ways for phase correlation and modelling, such as constant offset and common mid-point. The line was extended by Irish and German groups across Ireland to the Shannon Estuary, having a total length of over 600 km, but here we present only preliminary results for northern England and adjacent marine regions. The results for this region differ radically from those of the earlier LISPB experiment[2] in that we have recognized a well-defined 6.15 km s^{-1} basement interpreted as pre-Caledonian metamorphic basement beneath most of the line at relatively shallow depth, and there is a well-defined mid-crustal refractor starting at approximately 16 km depth beneath the North Sea and northern England which is not recognized beneath the Irish Sea.

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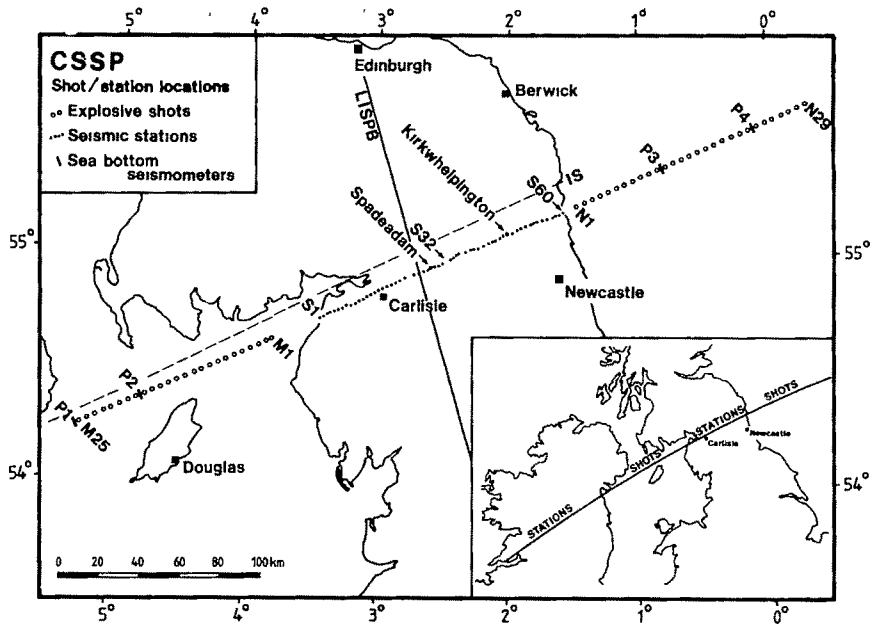


Fig. 1 Location of Caledonian Suture Seismic Project (CSSP), with details for Irish Sea to North Sea, showing also the supposed Iapetus suture[1] (IS) and the LISPB line[2].

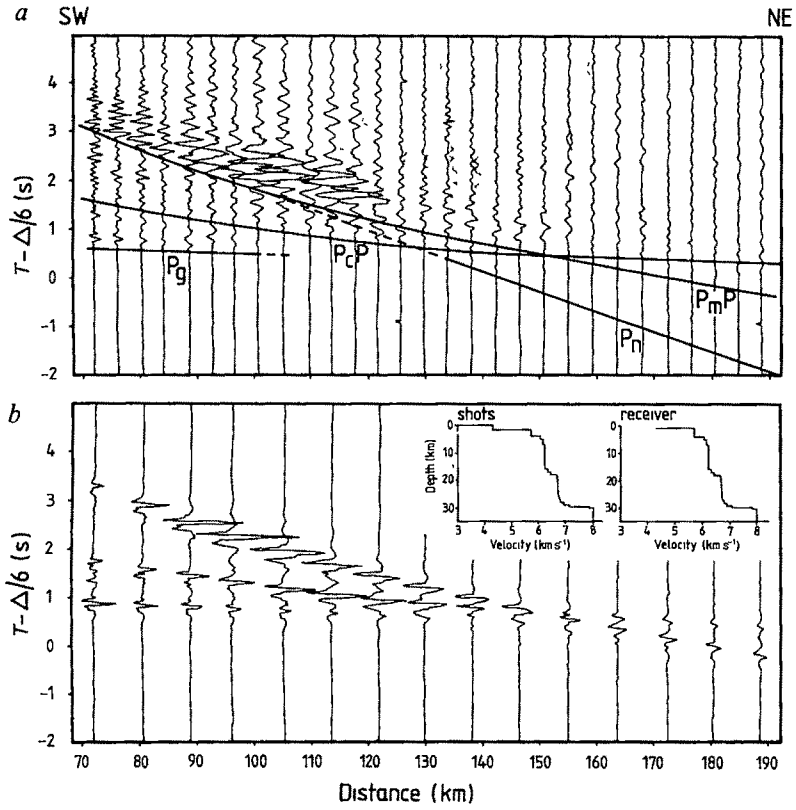


Fig. 2 *a*, Seismic recordings of North Sea shots N1–N29 at station S32 (Fig. 1) reduced to a velocity of 6.0 km s^{-1}, showing main phases computed for the synthetic model. *b*, Synthetic seismogram interpretation of *a* with inset velocity-depth distributions.

The following four main phases have been clearly recognized from the North Sea shots recorded in northern England, as illustrated by the recordings of the shots at station S32 (Figs 1, 2): (1) The first arrival beyond about 30 km is the P_g phase which has a velocity of 6.15 ± 0.02 m s^{-1} at 2–4 km depth as determined by time-term analysis. [The symbol P refers to compressional waves. P_g travels in the upper crust. P_cP and P_mP are wide-angle reflections or diving waves from respectively the mid-crustal discontinuity/gradient and the Moho. P_n is the Moho head wave refracted at the crust/mantle boundary.] P_g consistently dies out at all stations for shots beyond about N11–N13. Such decay of the P_g arrival has previously been cited as evidence for low-velocity zones in the upper crust. In the absence of laterally displaced sections containing similar offset distances, it has not been possible in past experiments to distinguish satisfactorily between the effects of a low-velocity zone and effects of lateral velocity variation in the upper crust. In this case, however, P_g is seen to decay in the vicinity of a specific shot position irrespective of station, so the cause must be interpreted as a lateral inhomogeneity near that shot position rather than as an underlying low-velocity zone. (2) A well-defined P_cP phase of apparent velocity ~6.5 km s^{-1} results from a steep increase in velocity with depth of ~6.22–6.5 km s^{-1} at 16–18 km depth, also detected in some earlier work[3,4]. (3) The P_mP phase representing the wide-angle reflection from the Moho is a clear large-amplitude arrival. (4) A small amplitude P_n phase of apparent velocity 7.9 km s^{-1} occurs as the first arrival beyond the crossover distance of 130 km. The P_mP and P_n phases are consistent with a sharp Moho at 30 km depth.

A model of the crustal structure assuming horizontal layering beneath the variable shallow structure, together with a synthetic seismogram computed using the reflectivity method[5], is shown in Fig. 2.

The Irish Sea shots recorded in northern England (Fig. 3)

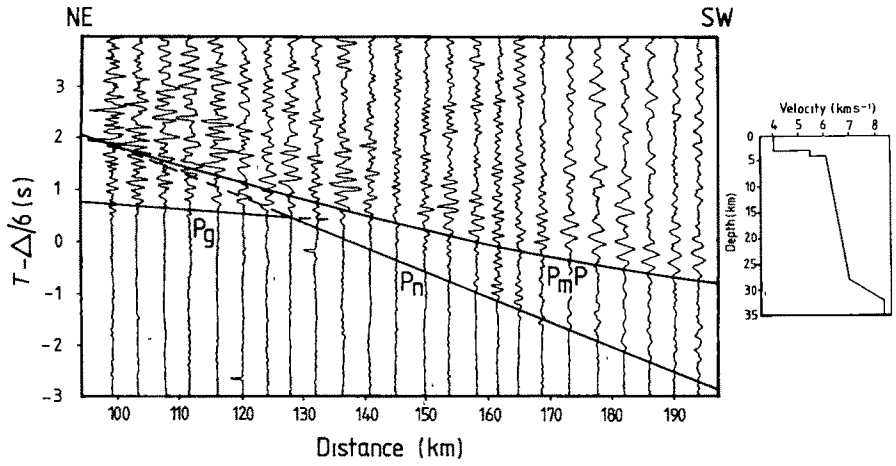


Fig. 3 Seismic recordings of Irish Sea shots M2–M25 at station S32 (Fig. 1) reduced to 6.0 km s^{-1}, showing the main phases computed by ray tracing for inset velocity–depth model.

yielded three main phases: (1) A P_g phase with a velocity of 6.15 ± 0.02 km s^{-1} determined by time-term analysis occurs as the first arrival between about 30 km and the crossover distance of 128 km, and comes from a depth of 2–4 km. Arrivals from shots over the Permo-Triassic basin beneath the Solway Firth are systematically delayed by the low-velocity sediments. (2) A conspicuous second arrival of 6.8–7.0 km s^{-1} apparent velocity is interpreted as the diving wave from a velocity gradient above the Moho at 28–32 km depth. (3) A P_n phase with an apparent velocity of 8.3 km s^{-1} or higher occurs as the first arrival beyond 128 km. The high apparent velocity is attributed to laterally varying time delays caused by the shallow sediment structure of the Solway basin.

A model of the velocity–depth distribution consistent with these three phases is shown in Fig. 3. A steep increase in velocity marking the top of a lower crustal layer, such as is indicated by the North Sea shots, is not apparent in the Irish Sea. Nevertheless, the possible existence of a higher-velocity lower crust, possibly underlying a velocity inversion at shallower levels, cannot be ruled out at this stage.

The shallow structure along the line of the experiment, which is probably just south of the Iapetus suture, is shown down to the 6.15 km s^{-1} basement in Fig. 4*a*. The structure down to the base of the Permo-Triassic was not resolved by the experiment and has been obtained from published sources[6] and from seismic reflection data supplied by Shell. The depth to the 5.5 km s^{-1} Lower Palaeozoic layer has been determined from first arrivals out to ~30 km range. The 6.15 km s^{-1} basement is well defined and its depth, as shown, has been inferred from time-term analysis.

The recognition of this 6.15 km s^{-1} upper crustal refractor at the relatively shallow depth of 4 km or less is a result of considerable significance. This layer extends down to about 16 km depth beneath the North Sea and Northumberland Trough, but its base is undetermined beneath the Irish Sea. The evidence indicates a slight increase in velocity with depth without obvious indication of a low-velocity layer within it. According to Hall *et al*[7,8], velocities >6.0 km s^{-1} at shallow depth beneath the Southern Uplands (north of the suture) represent crystalline metamorphic basement beneath the Lower Palaeozoic sequence rather than a metamorphic transition to greenschist facies within the Lower Palaeozoic[9]. Recordings along the Northumberland Trough of a land shot at Spadeadam (Fig. 1) indicate that the boundary between the 5.5–5.7 km s^{-1} Lower Palaeozoic rocks underlying the Carboniferous and the 6.15 km s^{-1} layer is sharp rather than gradational, which appears to rule out a metamorphic transition within the Lower Palaeozoic. The 6.15 km s^{-1} velocity is too low for oceanic crust. Both the velocity and the aeromagnetic anomalies[10] along the line are probably too uniform for island-arc-type crust. The simplest interpretation is that the Lower Palaeozoic rocks are relatively thin just south of the Iapetus suture, where they are underlain by crystalline basement which is probably formed of Precambrian metamorphic rocks. The contact could be an unconformity or a tectonic feature. This interpretation differs radically from the LISPB profile[2] crossing our line at Spadeadam, which indicated a 5.8–6.0 km s^{-1} Lower Palaeozoic basement extending down to at least 15 km depth.

A second important result is that the deep crust appears to differ between the North Sea/Northumberland Trough region

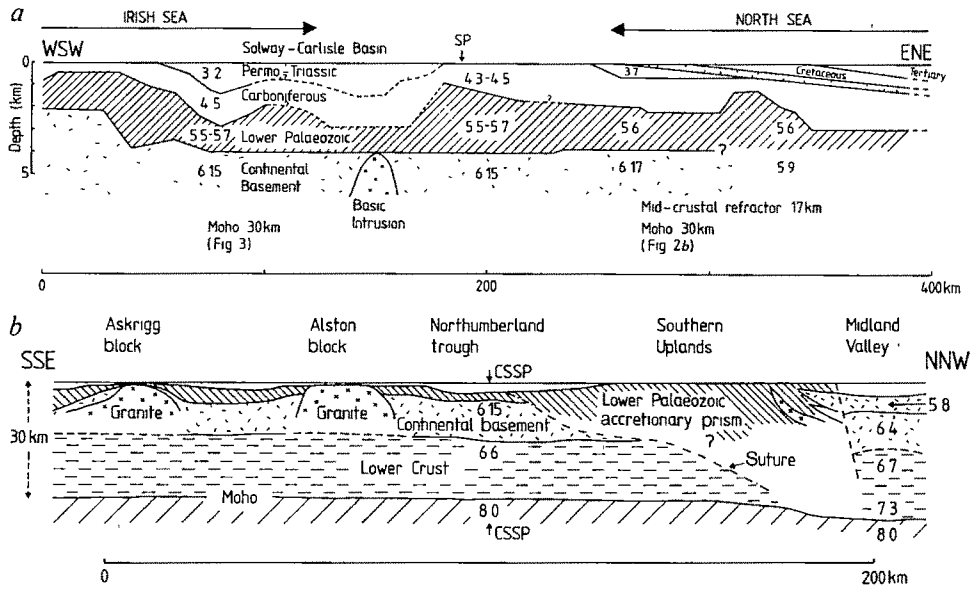


Fig. 4 *a*, Shallow crustal structure along the line of the experiment (Fig. 1), showing also the approximate locations of the crustal models of Figs 2*b* and 3. SP, Spadeadam. The basic intrusion beneath the Carlisle basin is inferred from the aeromagnetic map[10]. Seismic velocities in km s^{-1}. *b*, Speculative cross-section perpendicularly crossing the line of the experiment (marked CSSP) from the Midland Valley to the Northern Pennines, showing the possible relationship of the crust south of the Iapetus suture to the Southern Uplands accretionary prism. Seismic velocities are shown in km s^{-1} for this experiment (CSSP) and for the Midland Valley (LISPB[2])

and the Irish Sea region. P_cP has not been unambiguously detected beneath the Irish Sea, in contrast to the excellent North Sea development, and the Moho is interpreted as being gradational beneath the Irish Sea but relatively sharp beneath the North Sea. Taken at face value, this indicates juxtaposition of different crustal types along strike just south of the suture This conclusion, however, should be treated with some caution. The low-frequency P_cP and P_mP phases (Fig. 2) are most conspicuously developed for the shots in deeper water in the North Sea, which have significant energy in the 3-5-Hz band. It is possible that the lack of detection of P_cP for the Irish Sea shots, and the weaker P_mP development, may stem from the observed lack of significant energy in this band from the shallower Irish Sea shots. Future work aims to distinguish between these possibilities and to study transition between the two crustal types.

Comparison of our results with the relevant part of the WINCH crustal seismic reflection line[11] down the west of Britain is of interest. The WINCH line crosses the western extremity of our Irish Sea shot line, and a short section of WINCH was shot along the western end of our line to allow comparison between the vertical incidence and wide-angle surveys. A conspicuous feature of the WINCH data in the vicinity of our line is the concentration of strong sub-parallel and almost horizontal reflectors which occur at two-way travel times of 5-10 s, contrasting with the much more transparent regions above and below. The lower limit of these reflectors appears to correspond with the Moho, but from the present preliminary interpretation we have not been able to identify a velocity-depth signature beneath the Irish Sea corresponding to the upper boundary of the zone. It is possible that the distinctive 6.5 km s^{-1} lower crust at the North Sea end of our line corresponds to this zone of reflectors, which is well developed elsewhere on the mainland of England[12], but this needs future testing.

In conclusion, Fig. 4*b* is a speculative section showing our interpretation of how the crustal structure inferred from this experiment may relate to the Southern Uplands accretionary prism[13] to the north and the deep structure of the Pennine region[4] to the south The velocity-depth distribution beneath the Midland Valley comes from LISPB[2] and the Iapetus suture itself has been inserted in the form suggested by the WINCH line[11] further west. The relationship of the Lower Palaeozoic accretionary prism to the overthrust southern crust explains the observation of a 6.5 km s^{-1} lower crust beneath Eskdalemuir in the Southern Uplands[3] and why a higher velocity lower crust was not detected by LISPB[2].

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1 Phillips, W L A, Stillman C J & Murphy, T *Jl geol Soc. Lond* 132, 579-609 (1976)
2 Bamford, D, Nunn, K, Prodehl, C & Jacob, B *Geophys J R astr Soc* 54, 43-60 (1978)
3 Jacob, A. W B *Geophys J R astr Soc* 18, 189-197 (1969)
4 Bott, M H P, Swinburn, P M & Long, R E. *Proc Yorks geol Soc* 44, 479-495 (1984)
5 Fuchs, K. & Müller, G *Geophys J R astr Soc* 23, 417-433 (1971)
6 Day, G A *et al.* in *Petroleum Geology of the Continental Shelf of North West Europe* (eds Illing, L. V & Hobson, G D) 76-84 (Institute of Petroleum, London, 1981)
7 Hall, J *et al. Nature* 305, 418-420 (1983)
8 Hall, J *et al Nature* 309, 89-90 (1984)
9 Oliver, G J H & McKerrow, W S *Nature* 309, 89 (1984)
10 *1 250000 Series Aeromagnetic Anomaly Maps* (Institute of Geological Sciences, London)
11 Brewer, J A *et al. Nature* 305, 206-210 (1983)
12 Whittaker, A & Chadwick, R A *Geol Mag* 121, 621-624 (1984)
13 Leggett J K, McKerrow, W S & Eales M H *Jl geol Soc. Lond* 136, 755-770 (1979)

Hydrothermal manganese plumes in the Mid-Atlantic Ridge rift valley

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The concentration of dissolved manganese in sea water can be used as a chemical tracer of hydrothermal activity on the underlying sea floor. Here we present vertical profiles of seawater manganese concentrations above the rift valley of the Mid-Atlantic Ridge (MAR). The values obtained indicate the presence of plumes that are presently active generated by hydrothermal vents in the rift valley. These plumes are confined to the rift valley and do not spill over into the adjacent deep ocean basins. Differences between them suggest that there are at least five hydrothermal sources along this 1,700-km-long section of mid-ocean ridge.

The 9 MAR sites (Fig. 1) were preselected on the basis of various indicators of either former or present hydrothermal activity including a magnetic signature characteristic of hydrothermal alteration of the basaltic layer of oceanic crust[1], excess ^3He in the water column and hydrothermal mineralization on the sea floor[2,3]. Manganese has been established as a sensitive tracer of hydrothermal emanations[4-8].

The analyses were carried out as part of an extensive regional survey by the NOAA ship *Researcher* of the MAR between the TAG site at 26°N and the Vema Fracture Zone at 11°N in August 1984. Manganese was measured on board ship by flameless atomic absorption after preconcentration with oxine[9] to give a detection limit of 0 05 nmol kg^{-1}, four times lower than manganese concentrations normally found in deep-ocean waters[10]. A portable Zeeman tungsten furnace atomic absorption spectrophotometer (model AAZ, Scintrex Corporation) was used.

Most manganese data for ocean waters have been reported as 'total dissolvable manganese' (TDM), that is, the amount of manganese measured in an unfiltered sample after acidification to *p*H 2 with hydrochloric acid[11]. TDM represents essentially all the manganese, both dissolved and particulate, except for possibly the most refractory component[9]. Selected samples were analysed for TDM. However, TDM analyses are unsuitable for survey work because samples must be stored at *p*H 2 to ensure that leaching of particles is complete. To overcome this problem, samples were analysed without prior acidification In this way, six samples could be analysed within <2 h after the samples had arrived on deck. The manganese measured before acidification will be referred to as 'total reactive manganese' or TRM.

Samples were collected in 5-l Niskin and 30-l Go-Flo bottles mounted on a rosette or strung on a hydrowire. Fourteen aliquots including eight from plumes in the rift valley were filtered before being analysed for manganese. The average coefficient of variation between the concentrations of these filtered aliquots and the corresponding TRM results was 3.8%. This exercise demonstrates that TRM represents mainly the dissolved fraction as defined by passing through 0.4-μm filters. The difference between TDM and TRM is the manganese released from particles by acidification to *p*H 2 or acid-soluble manganese (ASM). In deep Atlantic waters not affected by hydrothermal discharge, excess particulate manganese is <0.04 nmol kg^{-1} (ref. 12), below the detection limit of the method used here. Therefore, in deep waters not affected by hydrothermal activity or resuspension, TDM and TRM should be equivalent within our analytical precision, which they were at the control station (Fig. 2*a*).

Typically, a manganese-depth profile of the open ocean is similar to the one at Vents station 1 (Fig. 2*a*) over abyssal hills some 660 km west of the MAR (26°00.0′ N; 51°00.0′ W). This profile, a control with which to compare results from the rift valley, consists of a surface water maximum of 1.63 nmol kg^{-1} and deep-water values of 0 26 ± 0.08 nmol kg^{-1}; these results are

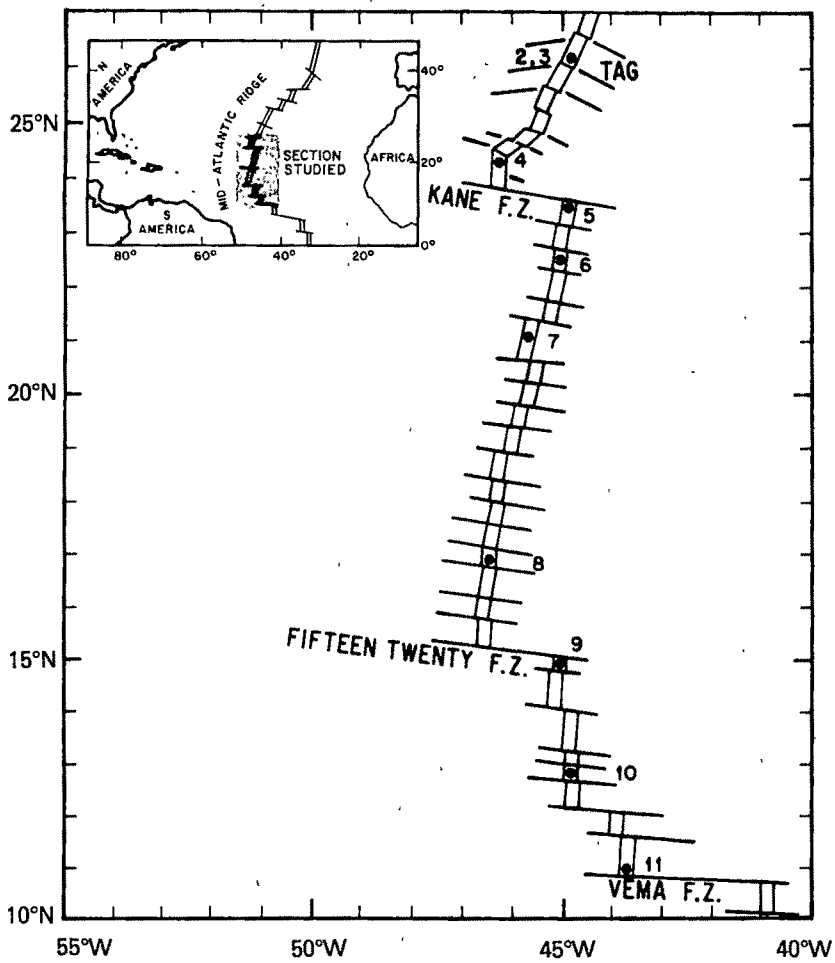


Fig. 1 Study area. Water sampling during the survey consisted of 11 stations: station 1, the control station west of the spreading centre, and stations 2–11 in the median valley of the MAR.

similar to data published for the nearby Nares Abyssal Plain[13]. Input from aeolian and riverine sources result in surface-water maxima at northern latitudes, but oxidation and scavenging maintain much lower concentrations in deep waters. Three processes may produce manganese anomalies below 1,000 m at open ocean sites: (1) diffusion from sediment after diagenetic remobilization; (2) resuspension of sediment by bottom currents; or (3) discharge of hydrothermal effluents. Diffusion from sediments is unlikely to be significant at our sites for two reasons. First, most deep-sea sediments are capped by an oxidizing layer that traps remobilized manganese within the sediment[14]. Second, there is very little sediment accumulation in the rift valley. Process (2) may produce an anomaly near the bottom, but such a signal is associated with particulate matter and thus would result in an anomaly in TDM but not TRM. In conclusion, the manganese anomalies discovered during this survey are most certainly produced by process (3).

Manganese is highly enriched in hydrothermal effluents because it is leached from basalt during hydrothermal circulation. The concentration of manganese in a hydrothermal end-member is ~1 mmol kg^{-1} (refs 15, 16), five million times greater than in deep ocean waters. Figure 2*b* is a TDM-depth profile over the vent field at Galapagos[4]. The buoyant hot-spring effluents emanating from the Galapagos Spreading Centre create a manganese anomaly that extends from the sea bed some 1,000 m into the water column. In contrast, the profile above the 'black smokers' at 21°N contains a manganese maximum 220 m above the sea floor (Fig. 2*c*)[6].

As well as the size and shape of the anomaly, the partitioning of manganese between dissolved and particulate phases provides useful information. Determining partitioning is useful because the manganese in reducing hydrothermal fluids is dissolved, but once discharged into oxygenated sea water, it converts to the particulate form as a result of oxidation and scavenging. Hence, the proportion of manganese present as particles is an indication of the age of the hydrothermal signal. Here, the difference between TDM and TRM (that is, ASM) will be used to infer distance from a source. However, we note that ASM is a function of time and not distance and, because both the kinetics of ASM formation and the directions of currents at our sites are unknown, such deductions should be considered tentative. Our results are presented in Table 1 and Fig. 3*a*–*i*.

Stations 2, 3 (TAG; Fig. 3*a*): Previous evidence for hydrothermal activity at the TAG site was substantial. The area is characterized by rapidly accumulating manganese crusts[17] and high metal contents in sediments[18]; temperature[19] and helium[20] anomalies in bottom waters have also been reported.

The manganese results from the TAG site show that there are active hydrothermal vents on the MAR, a conclusion supported by results from other sites occupied during the survey. Whether or not there are active vents at the TAG site is less clear. The manganese anomalies at station 2 are consistent with the presence of hydrothermal emanations from the east wall, as concluded from previous work at TAG[21]. If the anomaly is maintained by local activity, the largest sources probably occur at 3,450 m, somewhat deeper than previously thought. However, the results from TAG do not eliminate the possibility that the manganese anomaly here is advected from elsewhere along the MAR and that there is no present-day venting at this site. Indeed, the observation of large amounts of particulate manganese at station 2, L2, would support such a conclusion.

Station 4 (Fig. 3*b*): It is irrefutable that the excess manganese at this site is the result of hydrothermal activity. The shape of this profile is reminiscent of those found in the South Pacific[8] and could result from buoyant emanations from the valley floor, advection from the valley wall(s) near these locations or advection from vents some distance away. The small concentrations encountered and the observation that >20% of the anomalous TDM is associated with suspended particulates suggests that the anomaly here is an advected feature.

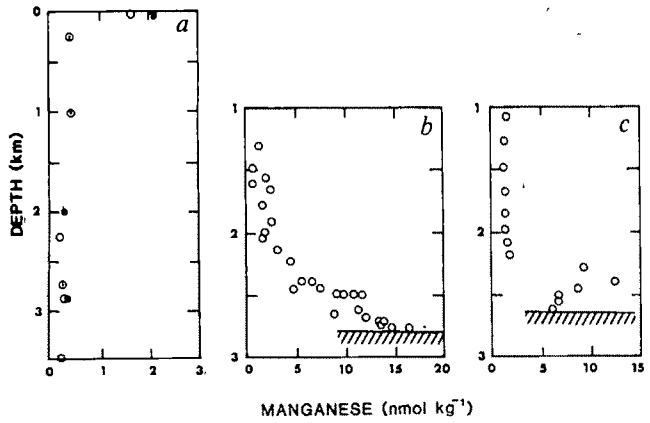


Fig. 2 *a*, Manganese-depth profile at the control station. Solid circles are total reactive manganese (TRM) and open circles are total dissolvable manganese (TDM). Circles with dots indicate identical concentrations within precision The bottom depth is ~5,000 m. *b*, TDM profile over the hot springs on the Galapagos Spreading Centre (redrawn from ref. 4). *c*, TDM profile over the 'smokers' on the East Pacific Rise at 21° N (redrawn from ref 6). The hatched lines in *b* and *c* indicate the bottom depth.

Station 5 (Fig. 3*c*): This station is in the rift valley at its eastern intersection with the Kane Fracture Zone (Fig. 1). Again, evidence for present-day hydrothermal activity was found. Whereas the size, shape and position of the anomaly here are similar to those at station 4, the form of the manganese is distinctly different; that is, there is very little particulate manganese (ASM) at this station, suggesting closer proximity to a source.

Station 6 (Fig. 3*d*): There is a distinct manganese anomaly at this station and very little ASM, indicating close proximity to active vents. The deepest sample has more particulate manganese, which may be explained by resuspension of sediments. The most obvious difference between the profile at station 6 and those further north is that the maximum concentration occurs much shallower, at 3,000 m as opposed to 3,500 m (although there is a small anomaly at 3,500 m as well).

Station 7 (Fig. 3*e*): The TRM anomaly at this location is the smallest found in the rift valley. There is 0.2–0.3 nmol kg^{-1} of particulate manganese in most samples as well, which may be related to the fact that the axis of the rift valley is anomalously offset ~30 km to the west at this site.

Station 8 (Fig. 3*f*): The TRM profile at station 8, L2, was obtained by positioning the rosette over the east wall at a depth as near as possible to 3,800 m. The most probable explanation for these results is that the rift valley anomaly (station 8, L1) is produced by advection of hydrothermal manganese from hot springs on the east wall at this site and that this venting occurs at a depth of 3,800 m. This tentative conclusion is based on four observations: (1) the maximum TRM concentration in the valley is 1.90 nmol kg^{-1}, compared with 2.58 nmol kg^{-1} over the wall; (2) these maxima occur at approximately the same depth in both profiles; (3) there is much more ASM in the valley, indicating that the signal there is older; (4) the shape of the east wall profile is similar to the one obtained over hot springs on the Galapagos Spreading Centre (Fig. 2*b*).

Station 9 (Fig. 3*g*): The results from station 8 suggested that the venting there may be asymmetrical about the spreading axis, that is, occurring on the east wall but not the west wall. To examine this problem more closely, two rosette lowerings were made at station 9. These results demonstrate that the distribution of hydrothermal manganese is asymmetrical at this site. Because the manganese plumes contain large quantities of ASM, both locations at this site may be somewhat removed from the nearest vents. The ASM results are similar to the concentrations of 'excess' manganese (0.6–1.0 nmol kg^{-1}) found by Lambert *et al.*[12] in the rift valley at 14°15′ N. The shapes and relative sizes of the anomalies suggest that the nearest venting is associated with the east wall and that the anomaly on the west wall is produced by westward advection.

Station 10 (Fig. 3*h*): The maximum in the valley occurs at about 3,600 m and is larger than the maximum over the east wall. These observations, together with the presence of more suspended particulate manganese at the east wall location, makes westward advection similar to that deduced for station 9 improbable. Indeed, the results are more compatible with a west wall source at this site.

Station 11 (Fig. 3*i*): This station was positioned in the rift valley at its western intersection with the Vema Fracture Zone. The TDM results demonstrate that the anomaly over the east wall is essentially all dissolved manganese. Given this evidence, station 11 must be considered as a promising site for more detailed investigation.

We conclude from the manganese plumes discovered during this survey that there are active hydrothermal vents along this section of the Mid-Atlantic Ridge. The differences between these plumes make it virtually impossible for them to have originated at the same vent field. First, they occur at different depths (2,900–3,800 m), over different ridge segments (Fig. 1) offset by as much as 150 km. Furthermore, the size and shape of the anomaly change, both across the rift and along the spreading

Table 1 Manganese results for MAR rift valley stations

Station	Lowering	Location	Bottom depth (m)	TRM maximum*				
				Depth (m)	TRM	TDM (nmol kg^{-1})	ASM	% + RM
2	1	26°09.7′ N; 44°48.1′ W	3,130	3,090	1.02	—	—	—
	2	26°09.7′ N; 44°46.6′ W	3,674	3,468	0.91	1.16	0 25	78
3	1	26°08 0′ N; 44°47.5′ W	2,617	2,597	0.46	—	—	—
	2	26°08.5′ N; 44°46.5′ W	2,800	2,786	0.47	—	—	—
4	1	24°22.5′ N; 46°14.2′ W	4,480	3,504	1 07	1.41	0 34	76
5	1	23°27.8′ N; 44°55.5′ W	4,075	3,475	1 28	1.15	—	100
6	1	22°28.5′ N, 45°03.3′ W	3,626	3,026	1.31	1.30	—	100
7	1	21°06 0′ N; 45°46 5′ W	4,232	3,324	0.83	0.93	0.10	89
8	1	16°47 7′ N; 46°26.6′ W	4,300	3,800	1.90	2 62	0.72	73
	2	16°48.0′ N, 46°23.0′ W	3,705	3,685	2.58	2 84	0.26	91
9	1	14°53.8′ N; 45°01.6′ W	3,798	2,873	0.54	0 67	0.13	81
	2	14°55 0′ N, 44°54.0′ W	3,079	2,879	1 80	2.35	0.55	77
10	1	12°55 0′ N, 44°49.0′ W	3,590	3,574	0.69	0 95	0.26	73
	2	12°55.3′ N; 44°50.5′ W	4,645	3,620	1 18	—	—	—
11	1	11°01 3′ N; 44°38.1′ W	4,165	3,627	2.02	2.00	—	100
	2	11°01.5′ N; 44°42.0′ W	4,992	—	0.67	—	—	—

* TRM, Total reactive manganese, TDM, total dissolvable manganese, ASM, acid-soluble manganese.

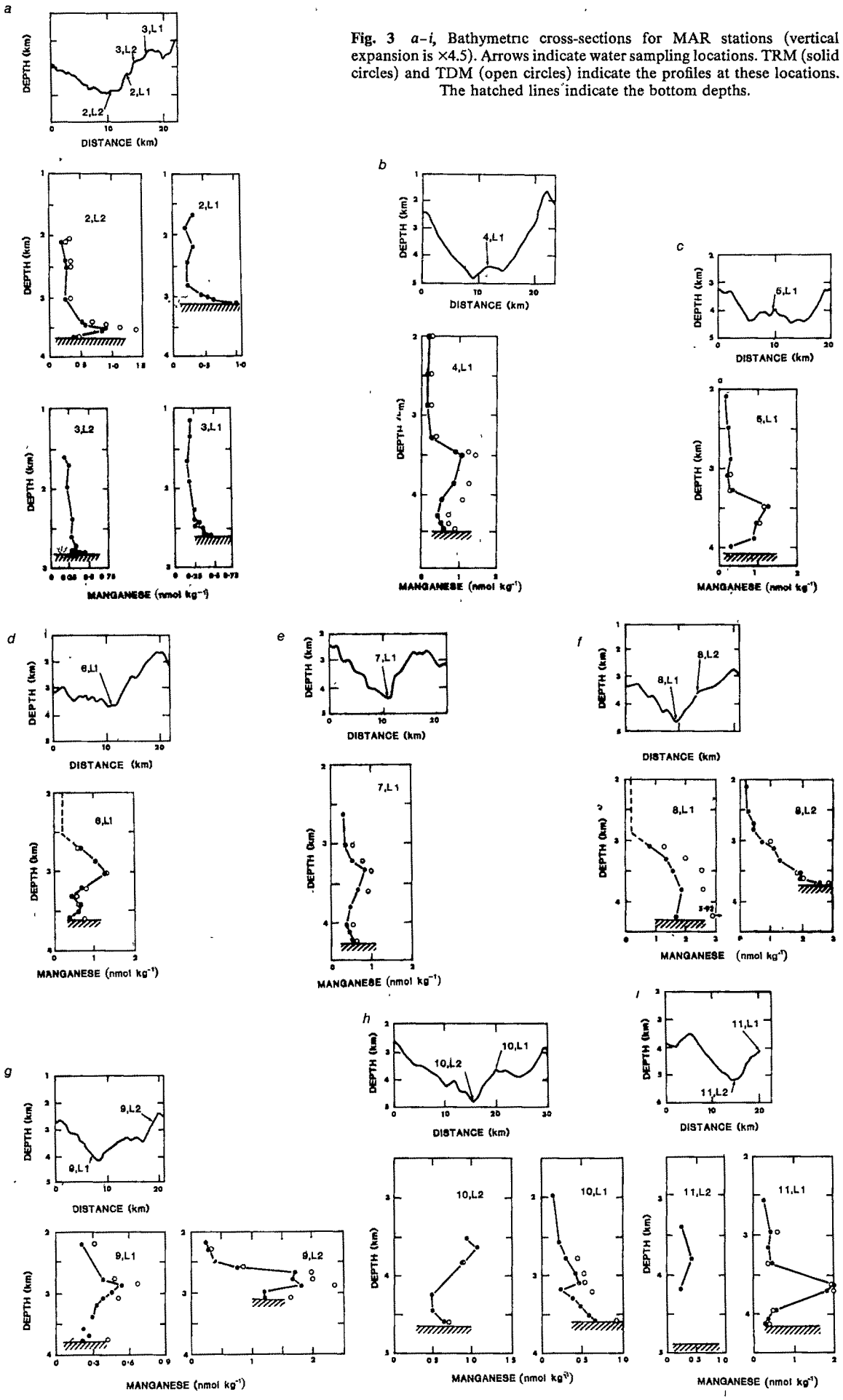


Fig. 3 *a–i*, Bathymetric cross-sections for MAR stations (vertical expansion is ×4.5). Arrows indicate water sampling locations. TRM (solid circles) and TDM (open circles) indicate the profiles at these locations. The hatched lines indicate the bottom depths.

axis. Finally, the proportion of the excess manganese associated with particles is variable.

By taking a conservative approach and grouping those plumes in which the maxima occur at the same depth, five sources can be identified, that is stations 2, 4 and 5; stations 6 and 7; station 8; station 9; and stations 10 and 11. This analysis predicts a frequency of one source per 340 km of ridge; of course the actual frequency may be much higher.

The position of venting in the rift appears to vary with latitude. Results from stations 8 and 9 are consistent with venting from the east wall, whereas results from station 10 indicate a west-wall source Furthermore, these results are consistent with discharge from the valley walls, but they do not eliminate the possibility that venting occurs from the valley floor. Finally, hydrothermal manganese along this section appears to be confined to the median valley and does not spill over into the adjacent ocean basins, although it may leak out through fracture zones.

The anomalies reported here are smaller than those found near venting in the Pacific[4-7]. However, this difference is an artefact of the regional sampling strategy of this survey and is not a true indication of the relative impact of venting from the MAR on geochemical cycles and the heat budget. Such information can be acquired only by site-specific work.

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1 Rona, P A *J Volcanol geotherm Res* **3**, 219-225 (1978)
2 Rona, P A *et al Geol Soc Am Abstr Progm* **14** (7), 602 (1982)
3 Rona, P A *Earth Sci Rev* **20**, 1-104 (1984)
4 Klinkhammer, G, Bender, M & Weiss, R F *Nature* **269**, 319-320 (1977)
5 Klinkhammer, G P *Chem Geol* **29**, 211-226 (1980)
6 Lupton, J E *et al Earth planet Sci Lett* **50**, 115-127 (1980)
7 Jones, C J, Johnson, H P & Delaney, J R *Geophys Res Lett* **8**, 873-876 (1981)
8 Klinkhammer, G, Elderfield, H & Hudson, A *Nature* **305**, 185-188 (1983)
9 Klinkhammer, G *Analyt Chem* **52**, 117-120 (1980)
10 Klinkhammer, G P & Bender, M L *Earth planet Sci Lett* **46**, 361-384 (1980)
11 Bender, M, Klinkhammer, G & Spencer, P *Deep-Sea Res* **24**, 799-812 (1977)
12 Lambert, C E, Bishop, J K B, Biscaye, P E & Chesselet, R *Earth planet Sci Lett* **70**, 237-248 (1984)
13 Burton, J D, Maher, W A & Statham, P J in *Trace Metals in Seawater* (eds Wong, C S, Boyle, E, Bruland, K W, Burton, J D & Goldberg, E D) 415-426 (Plenum, New York, 1983)
14 Froelich, P N *et al Geochim cosmochim Acta* **43**, 1075-1090 (1979)
15 Bischoff, J L & Dickson, F W *Earth planet Sci Lett* **25**, 385-397 (1975)
16 Edmond, J M, Von Damm, K L, McDuff, R E & Measures, C I *Nature* **297**, 187-191 (1982)
17 Scott, M R, Scott, R B, Rona, P A, Butler, L W & Nalwalk, A J *Geophys Res Lett* **1**, 355-358 (1974)
18 Shearme, S, Cronan, D S & Rona, P A *Mar Geol* **51**, 269-291 (1983)
19 Rona, P A *Geophys Res Lett* **5**, 993-996 (1978)
20 Jenkins, W J, Rona, P A & Edmond, J M *Earth planet Sci Lett* **49**, 39-44 (1980)
21 Rona, P A *J geol Soc, Lord* **137**, 385-402 (1980)

Fractal dimension of vegetation and the distribution of arthropod body lengths

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Following Mandelbrot[1], recent studies[2-6] demonstrate that some natural surfaces are fractal. Here we show that transects across vegetation are fractal, and consider one possible consequence of this observation for arthropods (mainly insects) living on plant surfaces. An important feature of a fractal curve or surface is that its length or area, respectively, becomes disproportionately large as the unit of measurement is decreased[1]. This suggests that if vegetation has a fractal structure, there is more usable space for smaller animals living on vegetation than for larger animals. Hence, there should be more individuals with a small body length than a large body length. We show that this is the case, and that relative numbers of small and large individual arthropods collected from vegetation are broadly consistent with theoretical predictions originating from the fractal nature of vegetation[7] and individual rates of resource utilization.

Mandelbrot[1,4] asks, how long is the coast of Britain? Measuring its length by stepping round with successively shorter divider step-lengths allows finer and finer resolution of the coastline; if the log of divider step-length is plotted against the log of total length measured, a straight line of negative slope is obtained[1,4,8]. Mandelbrot[1,4] interprets this as evidence that coastlines are fractal and that the slope of the graph is an estimate of $1-D$, where D is the fractal dimension of the coastline. The relationship between D, step-length r and the perceived length of the line $l(r)$ measured with step r is given by

$$l(r) \propto r^{(1-D)} \quad (1)$$

By definition[1], for a line (that is, a transect across a surface), D must lie in the range $1 \leq D \leq 2$ and for a surface, $2 \leq D \leq 3$.

The fractal dimension of vegetation might also be measured by stepping along a transect over the surface with a pair of dividers set to a series of step-lengths and using equation (1). This was the method used to measure the fractal dimension of transects across a coral reef[6]. An alternative widely used method (see ref 1, p. 130), which has some practical advantages, is as follows.

Enclose the object (or a subsection of it) in a square of side S and divide the square into $(S/\lambda)^2$ squares of side λ. Let $N(\lambda)$ be the number of squares the edge of the object enters and plot $\log N(\lambda)$ against $\log 1/\lambda$. Suppose that for small values of λ, the graph is almost linear with slope D. Then D can be interpreted as the fractal dimension, since

$$\log N(\lambda) \approx D \log 1/\lambda + \log K \quad (2)$$

where K is a constant, whence

$$N(\lambda)\lambda^D \approx K \quad (3)$$

The method is explained in greater detail in Fig. 1 legend, together with an example.

Photographs were taken of a variety of plants during early spring. The plants were either leafless, just coming into leaf or evergreen. By careful choice of plant-parts and camera focal length, the resulting photographs were approximately two-dimensional, greatly simplifying their interpretation under the grid (Fig. 1*a*). Two different arbitrarily sized grids (128 mm or 256 mm along one edge) were partitioned into 2^n squares along each edge. Depending on the grid size, n varied from 2 to 6 or 7, down to a square size of 2 mm. The grid was randomly repositioned several times to give semi-independent estimates of the fractal dimension of the same piece of vegetation. The slope of the resulting line (for example, Fig. 1*b*) was estimated by regression analysis.

There are several points to note in the interpretation of such graphs. One is that when the number of divisions is small, it is probable that all the squares will be entered, hence the slope of the graph will be $D=2$. This is an artefact of the method; consequently, in the present study all points that fell on the line $y=2x$ (on a log/log plot) were omitted when estimating the slope of the line. At the other extreme of resolution, when a very large number of squares is used, the slope of the graph will in general fall to 1. This could be due to a lack of resolution in the photograph or because irregularities in the outline of the plant no longer occur at that scale. Taking a photograph at a much larger scale (higher magnification) resolves this problem. The graph may be convex[9,10] between the extremes of $D=2$ and $D=1$, or it could have two straight subregions characterized by different slopes (Fig. 1*b*). Similar changes in fractal

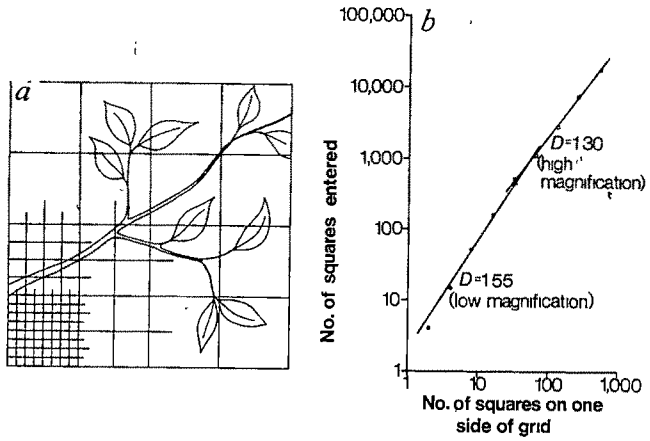


Fig. 1 *a*, Photographs of plants at various magnifications were placed under a grid. The number of squares entered by the outline of the plant were counted, starting with a course grid of two large squares on one side, then 2^n squares, with n varying from 2 to 6 or 7, depending on the grid size. For ease of representation, the plant's leaves in this figure are drawn flat; in reality they are orientated at all angles with respect to the grid. Also for clarity, the progressively finer divisions are only illustrated in one corner of the figure. The logarithm of the number of squares entered by the outline of the plant was then plotted against the logarithm of the number of squares along one side of the grid, as in *b*. The slope of the line equals the fractal dimension, *D*. *b*, Data gathered in this way for Virginia creeper, photographed without leaves in early spring. The twigs were photographed at one scale, then parts of the same twigs were rephotographed at a higher magnification, permitting *D* to be estimated at two levels of resolution.

dimension at different scales have been found in other natural objects[1,3,11].

Table 1 presents data on the fractal dimension of a selection of plants. Estimates of *D* range from 1.28, for a close-up photograph of Virginia creeper, to 1.79 for cotoneaster. Those plants which one might, *a priori*, consider to have a more complex growth form have a higher fractal dimension. In those plants which were photographed at two scales, estimates of the fractal dimension are lower at the higher magnification, as would be expected from the above argument.

The mean fractal dimension of the samples in Table 1 is 1.44. For ease of calculation, assume that, typically, $D \approx 1.5$. It follows from equation (1) that for an order-of-magnitude decrease in ruler length, the expected distance between two points on a linear transect (of dimension $D = 1.5$) across a fractal surface increases by a factor of $\sqrt{10} = 3.16$. The difficulties of measuring the fractal dimension of a surface are considerable and no data are yet available. However, squaring the increase in linear distance (that is, adding the fractal dimensions of the orthogonal transects; ref. 1, p. 365) gives a heuristic upper bound estimate for the expected increase in surface area. Therefore, the maximum expected increase in surface area is $3.16^2 = 10.0$ for an order-of-magnitude decrease in ruler length. Bearing in mind the disconnected character of the surface of vegetation, an estimate for the lower bound of the fractal dimension is obtained by adding 1 to the linear fractal dimension (for example, 1.5 + 1; see ref. 1, p. 365). This holds exactly under some circumstances, and in particular when the surface is flat in a direction transverse to the cross-section (which is clearly not the case for vegetation). This lower bound predicts a 3.16-fold increase in surface area for an order-of-magnitude decrease in ruler length, when $D = 1.5$.

Now consider the implications of the fractal nature of plant surfaces for the animals living on them. As a first approximation, substitute animal body length (L) for step-length (λ) in equation (1). If the way in which animals perceive and use their environment is proportional to their body length[12], then for a homogeneous fractal surface having transects with $D = 1.5$, the area perceived by animals 3 mm long may be up to an order of magnitude greater than the area perceived by animals 30 mm long, for the same reference area. This increase in available space for animals of smaller body length may be combined with a consideration of the way in which metabolic rate scales with body length[13,14] to make predictions about the distribution of body lengths of animals living on vegetation.

The metabolic rate of individual animals[13,14] scales approximately as the 0.75 power of body weight, W, that is, as $(L^3)^{0.75}$. Next, suppose that population densities are approximately proportional to the reciprocal of individual rates of resource utilization (that is, to metabolic rate^{-1}; see, for example, ref. 13). Then, if use of resources per individual is proportional to $W^{0.75}$, it follows that population density, N, scales as $(L^3)^{-0.75}$. Hence, all other things being equal (especially the rate of appearance of new resources), a 10-fold decrease in body length results in a $(10^3)^{0.75} = 178$-fold increase in the density of individuals. This increase in density may be combined with the expected increase in the available surface area, outlined above, for a given decrease in body length, to predict relative numbers of individual animals of different body lengths living on the surface of vegetation. For an order-of-magnitude decrease in body length, such calcula-

Table 1 Estimate of the fractal dimension, *D*, of woody plants for arbitrarily positioned transects across their surfaces

Species	Magnification	No. of estimates	Mean *D*	s d.
Barberry, *Berberis vulgaris* L. (evergreen)	High	3	1.46	0.018
	Low	3	1.43	0.042
Virginia creeper, *Parthenocissus tricuspidata* (Sieb. and Zucc.) Planch. (twigs and buds)	High	6	1.28	0.078
	Low	3	1.55	0.009
Weeping elm, *Ulmus glabra* forma *pendula* (Loud.) Rehd. (twigs and buds)	Low	6	1.41	0.111
Cotoneaster, *Cotoneaster horizontalis* Decaisne (twigs and leaves)	High	3	1.35	0.019
	Low	6	1.79	0.093
Ivy, *Hedera helix* L. (evergreen)	Low	18	1.39	0.050
Yew, *Taxus baccata* L. (evergreen)	High	3	1.47	0.042
	Low	3	1.68	0.099
Silver birch, *Betula pendula* Roth (twigs and leaves)	Medium	3	1.40	0.040
Downy birch, *Betula pubescens* Ehrh. (twigs and leaves)	Medium	3	1.40	0.035
Ash, *Fraxinus excelsior* L. (twigs and leaves)	Medium	3	1.42	0.083
Sycamore, *Acer pseudoplatanus* L. (twigs and leaves)	Medium	3	1.31	0.023

Photographs of branches and twigs of a selection of woody plants, with or without leaves, were taken from the University of York campus or Skipwith Common, North Yorkshire. High-magnification estimates were derived from close-up photographs of twigs ~10 cm long; low-magnification estimates were from photographs of twigs >50 cm long. Single medium estimates were made from specimens >25 cm long. The state of each plant when photographed and the plant part photographed is indicated in parentheses after the species name. (Note that sample transects inevitably tended to follow twigs and other 'principal axes' in the natural structure of the vegetation. This could introduce some bias into the estimates of their fractal dimension.)

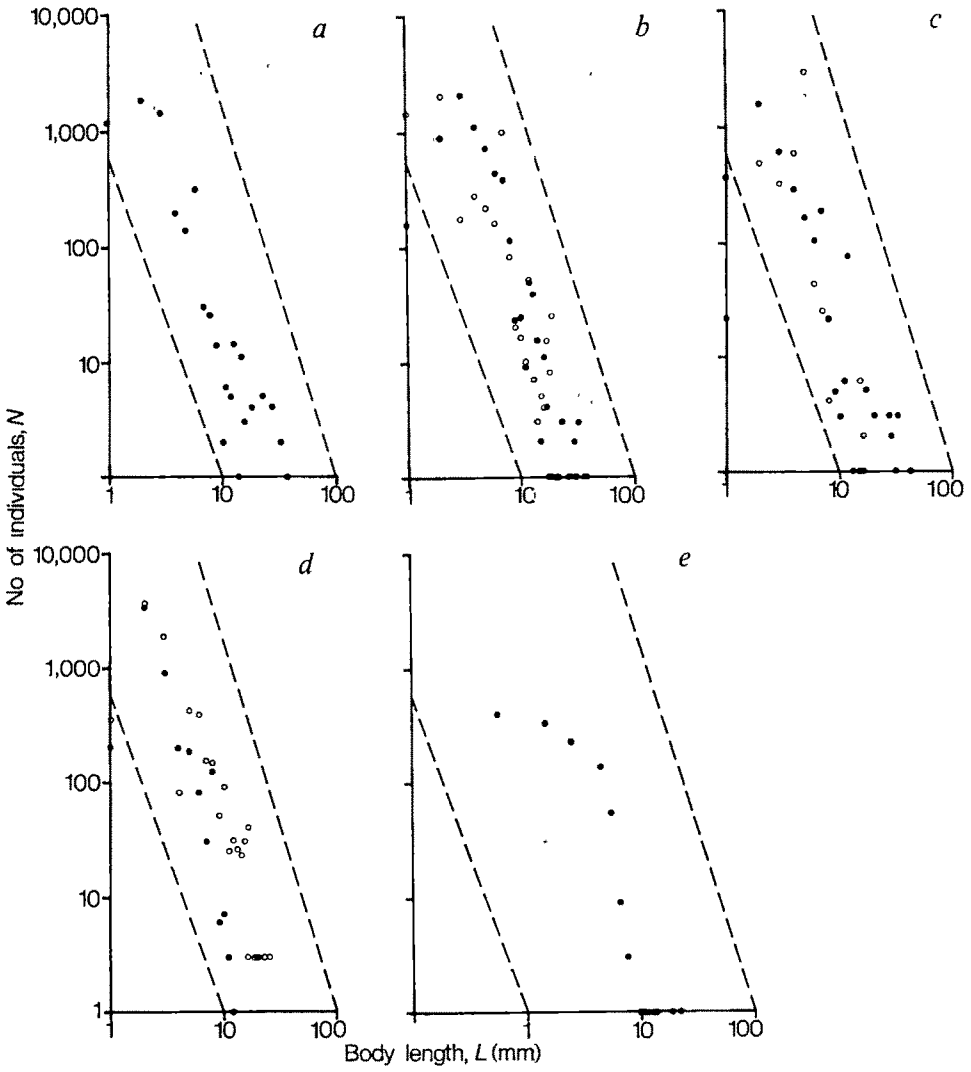


Fig. 2 Data on the number of individual arthropods (mainly insects) of different body lengths collected from vegetation. *a*, Understorey foliage in primary forest, Finca Tabogo, Costa Rica[15]; *b*, Osa secondary vegetation (●) and Kansas secondary vegetation (○); *c*, Tabogo primary riparian vegetation (●) and Icacos vegetation (○)[16]; *d*, understorey foliage in cacao plantations in Dominica (●) and at Finca La Lola, Costa Rica (○)[17]; *e*, Birch (*Betula pubescens*) trees at Skipwith Common, North Yorkshire. In *a*–*d*, arthropods were collected by sweep net, in *e* by pyrethrum knock-down of the tree canopy. The lower bound prediction that, for an order-of-magnitude decrease in body length, there should be a 560-fold increase in the number of individuals, is indicated by the lower dashed line on each graph; the upper bound prediction—a 1,780-fold increase for an order-of-magnitude decrease in body length—is shown by the upper dashed line. Regression lines were fitted through the log-transformed data, the anti-logarithm of the slopes of the lines were taken and are as follows: *a*, 390; *b*, 1,040 (●) and 700 (○); *c*, 360 (●) and 980 (○); *d*, 21,800 (●) and 530 (○); *e*, 1,440. The decline in the numbers of individuals of <1 mm body length may be due to a variety of factors[12], not least of which is the difficulty in sampling very small arthropods Consequently, when fitting regression lines to the data, in *a*–*d* the 1 mm body length data point and in *e* the first three data points were omitted.

tions predict an increase of between $178 \times 3.16 = 560$-fold and $178 \times 10 = 1{,}780$-fold in the number of individual animals[7].

Data on the number of individual animals of different body lengths collected from the surface of vegetation are scarce. Figure 2 shows such data for sweep-net samples of terrestrial arthropods[15–17] and original data collected by pyrethrum knock-down of a tree (*Betula pubescens*) canopy. All the data show a steep increase in the number of individuals for an order-of-magnitude decrease in body length, in general agreement with our predictions (Fig. 2).

The above heuristic calculations may be reversed, allowing prediction of the expected fractal dimension of the surface of vegetation, given the mean of the fitted slopes for the distribution of individuals' body lengths shown in Fig. 2. This calculation results in a prediction of the fractal dimension of the surface of vegetation of $D = 2.78$.

Clearly, approximate agreement between data and our predictions does not prove that the fractal nature of vegetation contributes to a steep increase in number of individuals as arthropods get smaller. Moreover, different values for D (1.28–1.79; Table 1) and the metabolic rate exponent (0.62–0.86; refs 13,14) lead to different predicted slopes in Fig. 2, as will the precise form of the relationship between the fractal dimension of a surface and transects across that surface. It remains to be seen whether detailed differences in the fitted slopes apparent in Fig. 2 can be explained by differences in the fractal dimension of particular plant surfaces, and by the biology of associated arthropods.

We know of no other attempts to explain patterns in the body size distributions of arthropods living on plant surfaces, although previous studies have examined species/frequency distributions for animals of different sizes[12,18]. Theoretical and empirical links between patterns in the number of species and number of individuals of different body lengths constitute an important unsolved problem.

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1 Mandelbrot, B B *The Fractal Geometry of Nature* (Freeman, New York, 1983)
2 Avnir, D, Farin, D & Pfeifer, P *Nature* **308**, 261–263 (1984)
3 Mandelbrot, B B, Passoja, D E & Paullay, A J *Nature* **308**, 721–722 (1984)
4 Mandelbrot, B B *Science* **156**, 636–638 (1967)
5 Burroughs, P A *Nature* **294**, 240–242 (1981)
6 Bradbury, D G, Reichelt, R E & Green, D G *Mar Ecol Prog Ser* **14**, 295–296 (1984)
7 Lawton, J H in *Plant Surfaces* (eds Juniper, B E & Southwood, T R E) (Edward Arnold, London, in the press)
8 Richardson, L F *Gen Syst* **6**, 139–187 (1961)
9 Paumgartner, D, Losa, G & Weibel, E R *J Microsc* **121**, 51–63 (1981)
10 Rigaut, J P *J Microsc* **131**, 41–54 (1984)
11 Orford, J D & Whalley, W B *Sedimentology* **30**, 655–668 (1983)
12 May, R M in *Diversity of Insect Faunas* (eds Mound, L A & Waloff, N) 188–204 (Blackwell, Oxford, 1978)
13 Peters, R H *The Ecological Implications of Body Size* (Cambridge University Press, 1983)
14 Schmidt-Nielsen, K *Scaling Why is Animal Size so Important?* (Cambridge University Press, 1984)
15 Janzen, D H & Schoener, T W *Ecology* **49**, 96–110 (1968)
16 Janzen, D H *Ecology* **54**, 659–686 (1973)
17 Andrews, R M *Breviora* **454**, 1–51 (1979)
18 Hutchinson, G E & MacArthur, R H *Am Nat* **93**, 117–125 (1959)

Education policy and the heritability of educational attainment

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Many workers assume that genetically determined differences in intellectual ability[1–5] will be influenced little by changes in educational policy or other environmental interventions[6,7]. Others[8,9], however, have suggested that increasing equality of educational opportunity will lead to an increase in the heritability of educational attainment. The resolution of this issue has been delayed until now because of the extremely large sample sizes which would be required[10]. Education data on twins and their parents, from the Norwegian twin panel[11,12], provide a unique opportunity to determine the impact on the heritability of educational attainment of the more liberal social and educational policies introduced in Norway after the Second World War[13]. As reported here, for individuals born before 1940 there is a strong effect of family background on educational attainment, accounting for 47% of the variance, though genetic factors account for an additional 41% of the variance. For females born after 1940 and before 1961, the relative importance of genetic (38–45%) and familial environmental (41–50%) differences changes very little. For males born during the same period, the broad heritability of educational attainment has increased substantially (67–74%), and the environmental impact of family background has correspondingly decreased (8–10%). For males, at least, having well-educated parents no longer predicts educational success, as measured by duration of education, independent of the individual's own innate abilities.

Adult twins from the Norwegian twin panel, a population-based register of like-sexed twins born in Norway between 1915 and 1961 and presently resident there, were asked, by mailed questionnaire, to report the duration of their own education (I, 0–7 yr; II, 8–9 yr; III, 10–12 yr; IV, over 12 years of education) and that of both their parents. Questionnaires were returned by one or both twins from 8,389 families. Each member of 4,608 twin pairs reported his own educational level. Although well-educated twin pairs were overrepresented in the sample of respondents, the sample obtained was more nearly representative of the adult population than the samples obtained in most volunteer studies[11]. Polychoric correlations[14] for educational attainment were computed between male and female monozygotic (MZ) and dizygotic (DZ) twin pairs, between the parents of twins, and between parent and offspring for twins born before 1940, during 1940–49 and during or after 1950 (Table 1). (We report correlations rather than variances and covariances[15], as we are not making any predictions about the effects of social policy on the variance in educational attainment.) Data from all the twins were used in computing the two-way contingency tables from which polychoric correlations between parent and offspring were calculated. Data for each member of a twin pair were treated as independent observations in computing the parent/offspring contingency tables. Data from only the first twin to report were used to compute the parent/parent contingency tables.

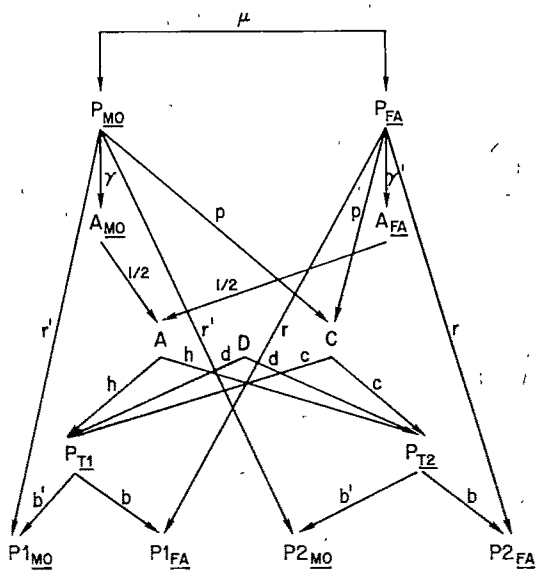


Fig. 1 Path diagram for MZ twins and their parents

We found that correlations between male and female MZ twins have changed little over the time period studied, implying that the total contribution of familial factors (both genetic and environmental) to variation in educational attainment has remained constant. The change in the correlation between parents (0.85 to 0.79), though statistically significant because of the very large sample sizes, is also very small. There is a slight decline in the correlation between female DZ twins for twins born after 1940 (0.75 to 0.68), but a much more pronounced decline for male DZ twins (0.77 to 0.48); such a decline, relative to the MZ correlation, would be predicted if the influence of genetic differences on educational attainment has become more important in twins born after 1939. A corresponding decline in the parent/offspring correlations also occurs, the change in the parent/son correlations being greater than the change in the parent/daughter correlations.

Combining self-report data from the twins with the education data which they report for their parents allows us to overcome many of the limitations of the classical twin design[15–17]. Provided that the effects of dominance or genotype × age interaction are not too great, this design will resolve the contributions to variation in educational attainment of additive gene action, the environmental effects of parental education, other environmental effects shared by twins or siblings which are unrelated to parental education, and the genetic consequences of assortative mating. We shall not, however, be able to resolve alternative

Table 1 Secular changes in correlations between twins and their parents

	Twins' year of birth 1915–39			1940–49			1950–60		
	n	*r*	s.e.	*n*	*r*	s.e.	*n*	*r*	s.e.
Male MZ twins	259	0.86	0.03	253	0.82	0.03	370	0 85	0.02
Female MZ twins	405	0.89	0.02	342	0.85	0.03	518	0.89	0 02
Male DZ twins	313	0.77	0.04	284	0.48	0.06	463	0.47	0.04
Famale DZ twins	425	0.75	0.03	400	0.68	0.04	576	0.66	0.03
Mother/son	1,652	0.74	0.02	1,456	0.61	0.03	1,809	0.51	0.02
Mother/daughter	2,001	0.72	0.02	1,694	0.57	0 03	2,179	0.55	0.02
Father/son	1,631	0.73	0.02	1,433	0,64	0 02	1,786	0 55	0.02
Father/daughter	1,971	0.74	0.02	1,660	0.79	0.02	2,121	0.60	0.02
Mother/father	2,519	0.85	0.01	2,027	0.79	0.02	2,542	0 79	0.01

Table 2 Correlations between educational levels of twins and educational levels reported by them for their parents

	Twin birth-cohort 1915-39						1940-49						1950-60					
	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)
Male MZ twins	(n=193)						(n=203)						(n=255)					
(1)	1 00	0 03	0 07	-	0.06	0 07	1 00	0 03	0 06	0 07	0.06	0.07	1 00	0 03	0 06	0 07	0.06	0 06
(2)	0 86	1 00	0 09	-	0.07	0.06	0.83	1 00	0.07	0.07	0 06	0 06	0 86	1 00	0 07	0 07	0 06	0 06
(3)	0.74	0 60	1 00	-	0 05	0 06	0.72	0 63	1 00	0 03	0 05	0 07	0 52	0 53	1 00	0 01	0 03	0 04
(4)	0 73	0 70	0 95	1 00	-	-	0 61	0 67	0 91	1 00	0 05	0.05	0 48	0 50	0 96	1 00	0 04	0 04
(5)	0 75	0 64	0 87	0 84	1.00	0 02	0 71	0 65	0 81	0 78	1 00	0.01	0 58	0 56	0 82	0 76	1 00	0 01
(6)	0 69	0 71	0 83	0 79	0 93	1 00	0 57	0 66	0 71	0 82	0 96	1 00	0 54	0 56	0 78	0 80	0.97	1 00
Female MZ twins	(n=310)						(n=284)						(n=361)					
(1)	1 00	0 02	-	0 06	0.06	0.06	1.00	0 03	0 06	0 07	0 05	0 05	1 00	0.02	0 05	0 06	0 05	0 05
(2)	0 90	1 00	-	0 06	0.06	0 06	0 84	1 00	0 07	0 06	0 07	0 06	0 89	1.00	0 05	0.05	0.04	0 05
(3)	0 80	0 71	1 00	-	-	-	0 63	0 49	1 00	-	0.04	0.06	0.55	0 58	1 00	0 01	0.03	0 03
(4)	0.74	0.72	0 98	1 00	0 04	0.04	0 61	0 64	0 95	1 00	0 05	0 05	0 50	0 58	0 97	1 00	0 03	0 03
(5)	0 71	0 65	0 88	0 85	1 00	0 01	0 68	0.48	0 83	0 72	1 00	-	0 61	0 65	0 83	0 77	1 00	0 01
(6)	0.67	0 67	0 79	0 86	0 96	1 00	0 70	0.63	0.71	0 78	0 96	1 00	0 56	0 65	0 77	0 82	0 95	1 00
Male DZ twins	(n=243)						(n=235)						(n=318)					
(1)	1.00	0.04	0 05	0 06	0 04	0 05	1 00	0 06	0 07	0 08	0 06	0 07	1 00	0.05	0 06	0 06	0 05	0 06
(2)	0 78	1 00	0 06	0 05	0 05	0 04	0.47	1 00	0.07	0.07	0.07	0 06	0 46	1.00	0 06	0 06	0 06	0 06
(3)	0 78	0 73	1 00	0.02	0 02	0 04	0 55	0 56	1 00	0 03	0 04	0.05	0 49	0.40	1 00	0 03	0 04	0 05
(4)	0.71	0.76	0 96	1 00	0 04	0 04	0 49	0.57	0 90	1 00	0 06	0 05	0 40	0 57	0 86	1.00	0 05	0.04
(5)	0 79	0 73	0 92	0.84	1 00	0 02	0 63	0 54	0 85	0 71	1 00	0 02	0 54	0.41	0 73	0.65	1 00	0 02
(6)	0 73	0 79	0 85	0 84	0 95	1 00	0 45	0 60	0 74	0 74	0 91	1 00	0.44	0.52	0.61	0 76	0 91	1 00
Female DZ twins	(n=309)						(n=319)						(n=406)					
(1)	1 00	0 04	0 05	0 06	0 05	0 05	1 00	0 04	0 07	0.07	0.06	0 06	1 00	0.04	0 05	0 05	0 04	0 04
(2)	0 78	1 00	0 05	0 05	0 05	0 04	0 72	1 00	0 06	0 06	0 05	0 05	0 69	1 00	0.05	0 05	0 05	0.05
(3)	0.75	0.75	1 00	-	0 03	0.05	0 49	0 60	1 00	-	0 05	0 05	0 56	0 46	1 00	0 01	0 03	0 03
(4)	0 65	0 76	0 96	1 00	0 05	0 05	0 39	0 62	0 95	1 00	0.05	0 05	0 48	0 52	0 94	1 00	0 04	0.03
(5)	0 74	0 77	0 89	0 80	1 00	0 01	0 57	0.64	0 76	0 69	1 00	0 01	0 60	0 49	0 83	0 70	1.00	0 01
(6)	0 73	0 80	0 81	0 77	0 96	1 00	0 47	0 65	0.72	0 75	0 96	1.00	0 60	0 56	0 78	0 81	0 91	1 00

Polychoric correlations are given in the lower triangle of each matrix, their standard errors in the upper triangle (1) First twin's education, (2) second twin's education, (3) mother's education (first twin's report), (4) mother's education (second twin's report), (5) father's education (first twin's report), (6) father's education (second twin's report) -, No estimate of standard error possible due to very low expected cell frequencies

hypotheses about mechanisms of mate selection and environmental transmission within families[18]. As we have separate reports of parental educational levels by each member of a twin pair, we can check the validity of such data.

Table 2 shows matrices of polychoric correlations, broken down by twin group and birth-cohort, between the educational levels of each twin, and the educational level reported by each twin for each parent; only twin pairs where each twin reported the educational levels of himself and both parents were used in computing these correlations. For some of the polychoric correlations in Table 2 no standard errors are given, because of very low expected frequencies for one or more cells of the two-way 4×4 tables from which the polychoric correlations are derived. Such coefficients may be strongly biased estimates of the true population correlations[14] and so were not used in any data analyses Agreement between twins is very good, correlations between educational levels reported by each twin for the same parent ranging from 0.86 to 0.98. However, the correlation between a twin's educational level and that which he reports for either parent is consistently higher than the correlation between his own educational level and the educational level reported by his co-twin for the same parent. Fortunately, we can allow for the effects of this bias, by model-fitting.

Figure 1 presents a path model[12,19] for deriving expected correlations between the educational attainments of MZ twins and the educational attainments reported by them for their parents P denotes true educational attainment (we assume that the twins make no error in reporting their own educational level), and P1 and P2 the educational attainments reported by first and second twins, respectively, for mother (subscript MO) or father (subscript FA). The standardized path regressions of true phenotypic value on additive genetic deviation (A), dominance deviation (D), familial environmental deviation (C) and random environmental deviation (E. omitted from the diagram to simplify presentation) are denoted by parameters h, d, c and e, or for females, in models which allow for sex-dependent effects, h', d', c' and e'. The path regression of additive genetic deviation on true phenotypic value is denoted by the parameter γ' in males, or γ in females, where $\gamma' = h + ac$, $\gamma = h' + ac'$, and a is the genotype/environmental correlation which may be expressed as a function of the other parameters of the model[3]. The path regressions of reported parental educational attainment on true parental educational attainment and on the twin's educational attainment are denoted by b and r for fathers, b' and r' for mothers Path regressions of offspring familial environmental value on true maternal or paternal educational attainment are denoted by the environmental transmission parameter p. The parameter μ denotes the correlation between the educational attainments of spouses, which is assumed to arise through phenotypic assortative mating[20]. An equivalent diagram can be drawn for DZ twins, and expected correlations derived

Models were fitted to the polychoric correlations in Table 2 by nonlinear weighted least-squares, using the standard errors of the polychoric correlations as weights, and treating the correlations as though they were independent. Strictly, these correlations are not independent. However, ignoring the correlation between correlations has been found to have little effect on the parameter estimates obtained[21]. Model-fitting yields estimates of model parameters and provides an approximate χ^2 value with which to assess the fit of a given model, and compare its fit with that of other models. The validity of the χ^2 statistic will depend on how closely the sampling distribution of the polychoric correlation coefficients approximates to the multivariate normal distribution With these very large sample sizes we expect the approximation to be close.

For twins born before 1940, there is no evidence for sex differences in the familial transmission of educational attainment A purely environmental model gives a good fit to the data ($\chi^2_{44} = 42\,18$, $P = 0.55$), although allowing for additive genetic variance gives a significant improvement in fit ($\chi^2_1 = 7.81$,

$P < 0.01$). Parameter estimates obtained for the best model are $h = 0.64$, $c = 0.53$, $\mu = 0.86$, $p = 0.19$, $r = 0.84$ *and* $b = 0.20$, so that 41% of the variance in educational attainment is attributable to additive genetic factors (h^2), 28% to the environmental effects of family background (c^2), and 19% to genotype/environmental covariation ($2hac$), the remaining 13% of the variance being attributable to environmental effects not shared by relatives. Our estimate of the importance of genotype/environmental covariation depends critically on the assumptions made about the mechanisms of environmental transmission and mate selection, which cannot be tested with these data. If we were to make certain alternative assumptions, no genotype/environmental covariation would be predicted[5] and the contribution of the family environment would be estimated at 47%.

For twins born after 1939, we find significant heterogeneity of gene expression and environmental effects across sexes. Purely environmental models are rejected with a high level of significance (1940–49: $\chi^2_{50} = 89.44$, $P < 0.001$; 1950–60. $\chi^2_{53} = 169.55$, $P < 0.001$). Models which allow for both additive gene action and environmental transmission from parent to offspring yield negative estimates for the environmental transmission parameter p, which suggests that any environmental effects of parents on their offspring are being masked by genetic dominance in this design. Models which allow for sex-dependent additive gene action, dominance and environmental effects shared by twins, but no environmental transmission from parent to offspring, give the best fits to these data (1940–49: $\chi^2_{48} = 52.61$, d.f. = 48; $\chi^2_{51} = 56.59$, d.f. = 51). Parameter estimates obtained for this model are, for twins born between 1940 and 1949: $h = 0.70$, $h' = 0.67$, $d = 0.50$, $d' = 0$, $c = 0.29$, $c' = 0.64$, $\mu = 0.72$, $r = 0.82$, $b = 0.31$; and, for twins born between 1950 and 1960: $h = 0.52$, $h' = 0.53$, $d = 0.63$, $d' = 0.32$, $c = 0.45$, $c' = 0.71$, $\mu = 0.73$, $r = 0.82$, $b = 0.40$. These estimates imply that genetic factors (including genetic dominance) account for 74% of the variance in educational attainment in male twins born in 1940–49, and 67% in male twins born in 1950–60, but account for only 45% of the variance in female twins born in 1940–49, and 38% in female twins born after 1949. Conversely, environmental effects shared by twins account for only 8% (1940–49) or 20% (1950–60) of the variance in males, but for 41% (1940–49) or 50% (1950–60) in females. As genetic dominance is a major source of variation in IQ[1–3,22], the absence of evidence for dominance in the pre-1940 sample might be interpreted as evidence against a major influence of IQ on educational attainment in individuals born before 1940. However, dominance may be masked by strong environmental transmission in these data.

The results presented here are clearly consistent with the hypothesis that the importance of genetic influences on educational attainment is subject to secular change. Other explanations of our findings seem implausible. Perhaps more pre-war monozygotic male twin pairs have been misclassified as dizygotic, thus inflating the pre-war dizygotic male correlation? The proportion of pre-war male twins who are dizygotic (45.3%) differs little from the proportion of dizygotic twins in the other twin cohorts (44.4–48.8%). Perhaps a 'special twin environment' effect[23] is involved? As there has been a progressive decrease in the influence of parental educational levels on offspring educational attainment (see Table 1), we would expect the difference between male MZ and DZ twin correlations to have declined rather than increased in recent years. The most likely explanation, confirming the hypothesis of Scarr-Salapatek[8], is that increased educational opportunity has led to an increased dependence of educational attainment on innate ability.

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1 Jinks, J. L. & Fulker, D. W. *Psychol. Bull.* 73, 311–349 (1970)
2 Jinks, J. L. & Eaves, L. J. *Nature* 248, 287–289 (1974)
3 Loehlin, J. C. *Behav. Genet.* 8, 415–426 (1978)
4 Rice, J., Cloninger, C. R. & Reich, T. *Behav. Genet.* 10, 73–92 (1980)
5 Rao, D. C. *et al. Genet. Res.* 39, 187–198 (1982)
6 Jensen, A. R. *Genetics and Education* (Methuen, London, 1972)
7 Jensen, A. R. *Educability and Group Differences* (Methuen, London, 1973)
8 Scarr-Salapatek, S. *Science* 174, 1223–1228 (1971)
9 Scarr-Salapatek, S. *Science* 174, 1285–1295 (1971)
10. Eaves, L. J. & Jinks, J. L. *Nature* 240, 84–88 (1972)
11 Magnus, P., Berg, K. & Nance, W. E. *Clin. Genet.* 24, 103–112 (1983)
12. Heath, A. C. *et al. Behav. Genet.* (in the press)
13. *Educational Statistics* (Central Bureau of Statistics, Oslo, 1978)
14 Olsson, U. *Psychometrika* 44, 443–460 (1979)
15 Eaves, L. J. *et al. Heredity* 41, 249–320 (1978)
16 Eaves, L. J. *J. R. statist. Soc. Ser. A* 140, 324–355 (1976).
17 Martin, N. G. *et al. Heredity* 40, 97–116 (1978)
18 Heath, A. C. & Eaves, L. J. *Behav. Genet.* 15, 15–30 (1985)
19 Wright, S. *Ann. math. Statist.* 5, 161–215 (1934)
20 Fisher, R. A. *Trans. R. Soc. Edinb.* 52, 399–433 (1918)
21 Rao, D. C. *et al. Behav. Genet.* 7, 147–159 (1977)
22 Bashi, J. *Nature* 266, 440–442 (1977)
23 Kamin, L. J. *The Science and Politics of IQ* (Wiley, New York, 1974)

The genetic basis of Haldane's rule

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'Haldane's rule', formulated by J. B. S. Haldane in 1922, states that: "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex" (ref. 1). His rule is now known to apply in mammals, lepidopterans, birds, orthopterans and dipterans[1–5]. In *Drosophila*, for example, Bock[6] cites 142 cases of interspecific hybridizations that produce one sterile and one fertile sex in the offspring, all but one of these crosses yielding sterile XY males and fertile XX females. Despite much speculation, however, the genetic basis of Haldane's rule remains unknown. Haldane himself rejected the simple explanation that males are innately more sensitive than females to the effects of hybridization because groups with heterogametic females (such as birds and butterflies) usually show female sterility in hybrids, so that heterogamety itself is the critical feature. He and others[1,2,4,7] suggested that heterogametic infertility or inviability in hybrids arises by a genetic imbalance between X chromosomes and autosomes. An alternative explanation[5,8,9] is that this syndrome is caused by a mismatch of X and Y chromosomes. Here I show that in the *Drosophila melanogaster* subgroup, Haldane's rule for fertility apparently arises from a genetic interaction between X and Y chromosomes and not from an imbalance between sex chromosomes and autosomes. This finding has important implications for understanding the evolution of interspecific reproductive isolation.

Haldane's original explanation for asymmetrical sterility or viability rests on the fact that homogametic hybrids have a complete haploid set of chromosomes from each parent, but heterogametic hybrids lack an X chromosome from one of the parental species. The latter hybrids may lack the gene products from both autosomes and sex chromosomes of a complete haploid set which are thought to be necessary for hybrid fertility or viability. The genetic implications of this model are that epistatic interactions between conspecific X chromosomes and autosomes are necessary for proper gene action[7,10]. Alternatively, different species may differ in their linkage relationships; some loci present on the X chromosome of one might be carried on the autosomes of the other. Homogametic hybrids would then have the normal dose of each locus, while heterogametic hybrids would suffer from duplication and deficiencies[11].

One way to test this X-autosome imbalance theory is to produce interspecific hybrid females having both X chromosomes from a single parental species. Because such females will have the same degree of X-autosome mismatch as that of sterile males, they would be expected to be sterile. Only in three species of *Drosophila* (*D. simulans*, *D. mauritiana* and *D. sechellia*) is it possible to carry out such a test, as the three species will hybridize to produce fertile females and sterile males[12–15] and there exists in *D. simulans* a stock with attached X chromosomes. *D. simulans* is cosmopolitan, and *D. mauritiana* and *D. sechellia*

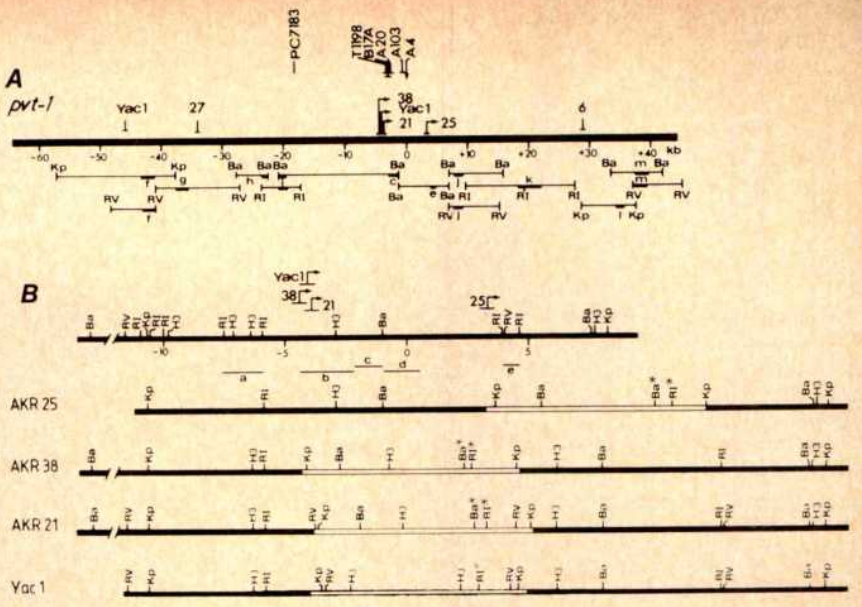


Fig. 1 The *pvt*-1 locus on chromosome 15. *A*, The cloned 108-kb region across the *pvt*-1 locus showing 6; 15 breakpoints in six plasmacytomas (top) and insertions in six T lymphomas; arrows indicate the probable transcriptional orientation of the proviruses, where known. A 17-kb region around the ABPC 4 6; 15 breakpoint is described in ref. 16; complete restriction maps of the clones defining the rest of the locus, the mapping of the four other plasmacytoma 6; 15 breakpoints, and the location of the PC7183 breakpoint described by Van Ness *et al.*[20] within this locus are described elsewhere[17]. Below the bar, the digests and lettered unique-sequence probes (thicker lines) used to survey this region are indicated. An ~1-kb region at +28 kb on the map could not be surveyed with the available probes. Abbreviations for restriction endonuclease sites are: RI, *Eco*RI; Ba, *Bam*HI; RV, *Eco*RV; H3, *Hin*dIII; and Kp, *Kpn*I. *B*, Proviral insertions within the *pvt*-1 locus. This expanded map of the region around the ABPC 4 breakpoint shows the location and orientation (arrows) of proviral inserts in T lymphomas; the bar indicates the possible error (±0.3 kb) in placement of the insertion points and an 8-kb gap has been introduced to save space. Restriction maps for four T lymphomas shown underneath the bar indicate the proviral inserts (open bars); the maps are incomplete, having been deduced by Southern blot analysis of genomic DNA. The *Eco*RI and *Bam*HI sites marked with an asterisk are markers for an MCF *env* region (see text). The probe fragments used for Southern blot analysis were derived from the following digests of cosmid or phage clones (see ref. 17): a and e, EcoRI; b, *Xba*I; c, *Xba*I + *Bam*HI: d, *Bam*HI + *Bgl*II; f, *Bam*HI + *Eco*RI; g, *Kpn*I; h, *Hin*dIII + *Bam*HI; i, *Bam*HI + *Xbaa*I; j, *Kpn*I; k, *Xho*I + *Kpn*I; l, *Xho*I + *Eco*RI; m, *Kpn*I.

not shown). The AKR 25 site, on the other hand, mapped between *c* and *e*. The AKR 25 rearrangement clearly resulted from an insertion, as probes from either side of the recombination site (*c* and *e*) hybridized to the same large rearranged *Hin*dIII fragment (shown for probe *c* in lane 9 of Fig. 2*A*); its size (~19.5 kb) compared with the germline fragment (10.6 kb) is consistent with insertion of ~9 kb of DNA lacking a *Hin*dIII site. Data obtained for the other three lines using flanking probes are consistent with the presence of a 9-kb insert bearing one *Hin*dIII site and one *Eco*RI site. Additional results from *Bam*HI, *Eco*RV and *Kpn*I digests allowed us to deduce the maps shown in Fig. 1*B*. The number of retroviral inserts in these tumours (see below) precluded using retroviral probes to verify that the inserts are proviruses, but all the data are consistent with that conclusion. The size of the inserts is that of a full-length (replication-competent) provirus, and the *Kpn*I sites found near both ends of each insert are almost invariant markers of murine proviral long terminal repeats (LTRs). The *Kpn*I (and *Eco*RV) sites associated with LTRs allowed us to localize the inserts more precisely. The AKR 38, AKR 21 and Yac-1 inserts fall within a ~1-kb region located only ~1 kb from three of the 6; 15 translocation breakpoints, while the AKR 25 insertion site lies 3 kb to the right of the ABPC 4 breakpoint (Fig. 1*A*). Thus, four retroviral inserts and five plasmacytoma breakpoints map within an 8-kb region.

Significantly, the four inserts have features characteristic of the MCF proviruses strongly implicated in leukaemogenesis[24]. Like most MCF inserts found near c-*myc*[7], all four bear an *Eco*RI site ~2 kb from one end and the three AKR inserts have a nearby *Bam*HI site (Fig. 1*B*). These hallmarks of the *env* region (encoding the glycoprotein region gp70) of a leukaemogenic MCF virus are acquired by recombination with endogenous non-ecotropic proviruses[24,27-30]. The hybrid gp70 is thought to promote leukaemogenesis by permitting retroviral invasion of thymocytes and/or providing them with a mitogenic stimulus[24]. As the *env* gene lies downstream of the viral *gag* and *pol* genes, all four proviral inserts would be transcribed from left to right on the maps in Fig. 1.

The three other rearrangements lie farther from the plasmacytoma breakpoints (Fig. 1*A*); although we lack sufficient unique-sequence probes in the relevant flanking regions to characterize them extensively, it seems likely that they also reflect proviral integration. The putative insert in AKR 6 maps ~29 kb to the right of the ABPC 4 translocation site (Fig. 1*A*), assuming that the acquired *Kpn*I site (Fig. 2*B*) lies within an LTR, while that in AKR 27 maps ~16 kb to the left of the PC7183 translocation breakpoint. Curiously, Yac-1 appears to have an additional insert still farther from the PC7183 site (Fig. 1*A*). The apparent presence of two inserts within *pvt*-1 in Yac-1 may reflect selection for further insertions during its long passage history.

Retroviral insertion within *pvt*-1 is unlikely to be coincidental, as insertion is thought to be random[31] and the 100-kb region surveyed here represents only 3.7×10^{-5} of the genome. Given ~10 inserts per AKR tumour (ref. 28 and our unpublished results), the chance of finding a single random insert within that

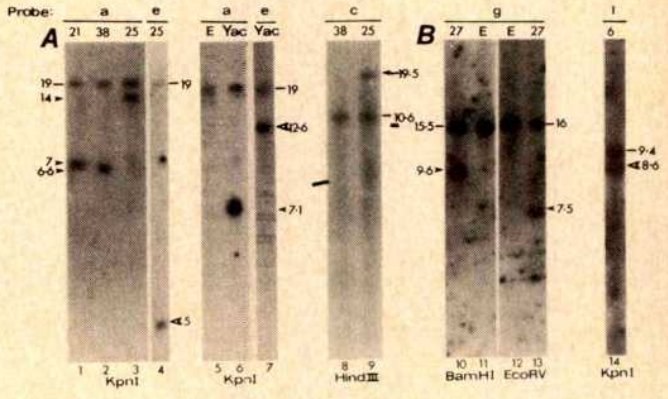


Fig. 2 Southern blots revealing *pvt*-1 rearrangements in T lymphomas. *A*, Rearrangements in the region favoured for 6; 15 translocations; *B*, more distant rearrangements. E denotes AKR embryonic DNA. Fragment sizes are in kilobases (kb). Bars indicate fragments from the unrearranged allele; solid arrowheads mark left-hand host–viral junction fragments, while open arrowheads indicate right-hand junction fragments. The *Bam*HI fragment analysed in lanes 10 and 11 spans from ~−42 to −28 kb on the map in Fig. 1*A*. Letters denote probes described in Fig. 1 legend. The methods used for preparation of DNA of high relative molecular mass, its digestion by the indicated restriction endonucleases, subsequent electrophoretic fractionation, hybridization and autoradiography have been detailed elsewhere[21,26].

region in the 33 tumours analysed is $\sim 10 \times 33 \times 3.7 \times 10^{-5} = 0.01$, and the odds of finding seven inserts by chance are $\sim (10^{-2})^7 = \sim 10^{-14}$. The possibility that *pvt*-1 is merely a preferred integration region, unrelated to oncogenesis, seems unlikely because chromosome 15 has not acquired endogenous proviruses[32], nor is *pvt*-1 merely a recombination 'hotspot', because translocations involving this band have not been associated with non-lymphoid tumours. Hence, it seems likely that the insertions contributed to T-lymphoma development. Whether *pvt*-1 is related to any of the previously identified retroviral insertion regions[11-13] is not yet clear; it does not correspond to *pim*-1, a common locus of M-MuLV insertion in murine T lymphomas[13], because their restriction maps differ markedly and *pim*-1 does not map to chromosome 15 (A. Berns, personal communication). Conceivably, *pvt*-1 may bear the murine homologue of one of the three common proviral insertion loci identified in rat thymomas (*mlvi*-1, *mlvi*-2 and RMoInt-1)[11,12]. Both *mlvi*-1 and *mlvi*-2 have homologues on mouse chromosome 15 (refs 14, 15), but that of *mlvi*-2 does not appear to map to the distal region of chromosome 15 (ref. 14), where *myc* and *pvt*-1 lie[4,18,19].

As the *pvt*-1 breakpoints lie at least 72 kb from the c-*myc* promoters[17], it is conceivable that *pvt*-1 bears another oncogene, but we have found no evidence that it bears sequences related to known oncogenes[17]. In particular, the putative mammary oncogene *int*-1 (ref. 33) and the c-*sis* gene, which both map to chromosome 15 (refs 33, 34), were not detected within the entire cloned *pvt*-1 (or c-*myc*) regions; we found no rearrangements near *int*-1 or *sis* when we screened *Eco*RI digests of the 32 AKR tumour DNAs with *int*-1 probe *c* (see ref. 33), provided by Drs H. Varmus and T. Fung, and a v-*sis* probe.

To determine whether *pvt*-1 is transcriptionally active, we used probes from the region near the major insertion/breakpoint region (from −11 kb to +4.8 kb on the map in Fig. 1*B*) to examine blots of poly(A)$^+$ RNA from several T lymphomas and plasmacytomas; only low levels of transcripts were found and none was consistently elevated (or depressed) in lines having *pvt*-1 alterations compared with those lacking any *pvt*-1 rearrangement (ref. 17 and J.A., unpublished results). Thus we have not yet found an attractive candidate for a *pvt*-1 gene that might be a target for the insertions and translocations.

Is retroviral insertion within the *pvt*-1 locus associated with active c-*myc* expression, as are the variant (6; 15) plasmacytoma translocations involving *pvt*-1 (refs 17, 19) and the variant Burkitt lymphoma translocations[35-39]? To address this question, we compared messenger RNAs from four T-lymphoma lines having *pvt*-1 inserts, six AKR lines having inserts near c-*myc*[7] and four AKR lines lacking detectable inserts within either locus. Nearly all the lines had comparable levels of the canonical ~2.4-kb c-*myc* mRNA (Table 1, Fig. 3). These levels fall within the range found in plasmacytomas bearing either the 6; 15 or 12; 15 translocation[17,19,40-44] (illustrated respectively by ABPC4 and TEPC609 in Fig. 3) and are well above the range observed in normal lymphoid tissues or in most chemically induced T lymphomas examined previously[40]. These results suggest that c-*myc* is activated in most retrovirally induced T lymphomas. In tumours with a provirus close to *myc*[7-10], activation is probably mediated by the LTR enhancer, which frequently disrupts putative control sequences upstream from *myc*[7]. We speculate that insertions within *pvt*-1, and perhaps yet other loci, can also activate c-*myc*, either directly or indirectly (see below). Of the 35 retrovirally induced T lymphomas we have studied, 9 AKR tumours and 2 T lymphomas induced by Soule MuLV had insertions near c-*myc* (ref. 7 and Table 1), and 6 had insertions within *pvt*-1 (Fig. 1*A*).

Our results suggest that alterations of *pvt*-1 may be conducive to T-cell as well as B-cell neoplasia, perhaps by inducing constitutive c-*myc* expression. As cloned sequences from the *pvt*-1 and c-*myc* loci establish that *pvt*-1 alterations occur at least 72 kb from the c-*myc* promoters[17], how might the effect be conveyed over such large distances? The 6; 15 translocations couple the left-hand side of the *pvt*-1 locus to the C_κ enhancer[16,17,20] and the insertions presumably introduce the LTR

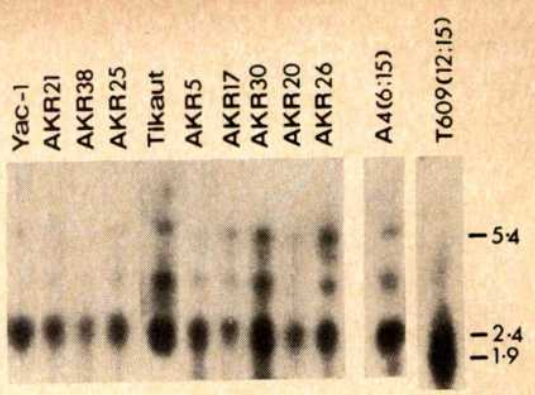


Fig. 3 Transcripts of the c-*myc* gene in 10 T lymphomas and 2 plasmacytomas. Blots were hybridized with a *myc* exon 3 probe (a *Sac*/*Xho* fragment)[41]. RNAs in the first four lanes derive from T lymphomas which have proviral inserts within *pvt*-1, while those in the second four lanes are from T lymphomas with inserts near c-*myc*[7]; AKR 20 and 26 have no inserts near c-*myc* or *pvt*-1 (Table 1). Plasmacytoma ABPC 4 (A4) has a t(6; 15) and contains the standard ~2.4-kb transcripts[17,19], while TEPC609 has the typical C_α-*myc* t(12; 15) fusion and yields only the 1.8–2.2-kb mRNAs derived from the rearranged gene[41]. The ~5.4-kb RNA is a nuclear precursor of the 2.4-kb mRNA[41]. Poly(A)$^+$ RNA was prepared by a method[45] involving lysis of tumour tissue in SDS and proteinase K, followed by oligo(dT)-cellulose chromatography. RNA was denatured by heating for 10 min at 60 °C in formaldehyde, and 3 μg was fractionated on a 1.5% agarose gel in formaldehyde and transferred to nitrocellulose[46].

Table 1 T-lymphoma insertions and c-*myc* expression

Tumour	c-*myc* locus*	*pvt*-1 locus†	c-*myc* mRNA‡
AKR 21	—	+	++
AKR 38	—	+	+
AKR 25	—	+	++
AKR 6	—	+	ND
AKR 27	—	+	ND
Yac-1	—	+	++
Tikaut	5′	—	+++
AKR 22	5′	—	++
AKR 12	5′	—	±
AKR 5	5′	—	+
AKR 16	5′	—	ND
AKR 17	5′	—	+
AKR 2	3′	—	ND
AKR 30	3′	—	++
AKR 14	3′	—	ND
AKR 20	—	—	++
AKR 19	—	—	++
AKR 26	—	—	++
AKR 10	—	—	+

Tumours denoted AKR arose spontaneously in mice 7–12 months old in our AKR mouse colony[7]. Tikaut is a cell line derived from an AKR tumour[27] and Yac-1 a cell line derived from a M-MuLV induced BALB/c tumour[22]. Tikaut is trisomic[25], but no cytogenetic data are available on the other lines.

* Insert detected within a 53-kb region across c-*myc* described elsewhere[17], extending from 27 kb 5′ to the c-*myc* promoters to 26 kb 3′ of them. All 5′ inserts map within 2 kb of the c-*myc* promoters[7]. The insert in AKR 30 maps ~24 kb 3′ of the c-*myc* promoters[7], and that in AKR 14 in the same vicinity (unpublished results), while that in AKR 2 appears to be in the 3′-untranslated portion of exon 3 (ref. 7).

† Insert detected within the 108-kb cloned region across *pvt*-1 (see Fig. 1*A*).

‡ Estimate of the amount of c-*myc* mRNA bases on several blots like that in Fig. 3; +++ indicates the level of mRNA in Tikaut; ++, about half that level; +, about one-quarter; and ±, about one-eighth. ND, not determined.

enhancer (and promoter), but enhancers are generally thought to act over distances of only 5–10 kb. Conceivably, the translocations/insertions may alter a *pvt*-1 gene that regulates *myc* expression, either disrupting a negative regulator or stimulating expression of a positive regulator, although we have not yet found a *pvt*-1 transcript that is consistently depressed or stimulated by the *pvt*-1 alterations. Alternatively, the effect may be conveyed along the chromosome (in *cis*) by disruption of long-range chromatin folding, although such effects might not be expected from a 9-kb insert. For either model, it is tempting to think that *pvt*-1 is involved in normal *myc* regulation.

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1. Klein, G. *Nature* **294,** 313–318 (1981).
2. Hayward, W., Neel, B. & Astrin, S. *Nature* **290,** 475–480 (1981).
3. Payne, G., Bishop, J. M. & Varmus, H. *Nature* **295,** 209–214 (1982).
4. Klein, G. *Cell* **32,** 311–315 (1983).
5. Perry, R. *Cell* **33,** 647–649 (1983).
6. Leder, P. *et al. Science* **222,** 765–771 (1983).
7. Corcoran, L., Adams, J. M. & Cory, S. *Cell* **37,** 113–122 (1984).
8. Li, Y., Holland, C., Hartley, J. & Hopkins, N. *Proc. natn. Acad. Sci. U.S.A.* **81,** 6808–6811 (1984).
9. Selten, G., Cuypers, H., Zijlstra, M., Melief, C. & Berns, A. *EMBO J.* **3,** 3215–3222 (1984).
10. Steffen, D. *Proc. natn. Acad. Sci. U.S.A.* **81,** 2097–2101 (1984).
11. Tsichlis, P., Straus, P. & Hu, L. *Nature* **302,** 445–449 (1983).
12. Lemay, G. & Jolicoeur, P. *Proc. natn. Acad. Sci. U.S.A.* **81,** 38–42 (1984).
13. Cuypers, H. T. *et al. Cell* **37,** 141–150 (1984).
14. Tsichlis, P., Straus, P. & Kozak, C. *Molec. cell. Biol.* **4,** 997–1000 (1984).
15. Tsichlis, P., Hu, L. & Strauss, P. G. in *UCLA Symposia: Normal and Neoplastic Hematopoiesis* (eds Golde, D. & Marks, P.) 399–415 (1983).
16. Webb, E., Adams, J. M. & Cory, S. *Nature* **312,** 777–779 (1984).
17. Cory, S., Graham, M., Webb, E., Corcoran, L. & Adams, J. *EMBO J.* (in the press).
18. Ohno, S. *et al. Cell* **18,** 1001–1007 (1979).
19. Ohno, S. *et al. J. exp. Med.* **159,** 1762–1777 (1984).
20. Van Ness, B. *et al. Nature* **301,** 425–427 (1983).
21. Cory, S., Gerondakis, S. & Adams, J. *EMBO J.* **2,** 697–703 (1983).
22. Gerondakis, S., Cory, S. & Adams, J. M. *Cell* **36,** 973–982 (1984).
23. Klein, E. & Klein, G. *J. natn. Cancer Inst.* **32,** 547–568 (1964).
24. Famalari, N. *Curr. Topics Microbiol. Immun.* **103,** 75–108 (1983).
25. Spira, J. *et al. Int. J. Cancer* **28,** 785–798 (1981).
26. Cory, S. *et al. EMBO J.* **2,** 213–216 (1983).
27. Chattopadhyay, S. *et al. Nature* **295,** 25–31 (1982).
28. Herr, W. & Gilbert, W. *J. Virol.* **46,** 70–82 (1983).
29. van der Putten, H. *et al. Cell* **24,** 729–739 (1981).
30. Kelly, M., Holland, C., Lung, M. & Chattopadhyay, N. *J. Virol.* **45,** 291–298 (1983).
31. Varmus, H. *Science* **216,** 812–820 (1982).
32. Kozak, C. in *Mechanisms of B Cell Neoplasia* (eds Melchers, F., Potter, M. & Weigert, M.) 126–133 (Roche, Basle, 1983).
33. Nusse, R., van Doyen, A., Cox, D., Fung, Y. & Varmus, H. *Nature* **307,** 131–136 (1984).
34. Kozak, C., Sears, J. & Hogan, M. *Science* **221,** 867–869 (1983).
35. Hollis, G. *et al. Nature* **307,** 752–755 (1984).
36. Taub, R. *et al. Cell* **37,** 511–520 (1984).
37. Davis, M., Malcolm, S. & Rabbitts, T. *Nature* **308,** 286–288 (1984).
38. Croce, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 6922–6926 (1983).
39. Erikson, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 7581–7585 (1983).
40. Adams, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 6966–6970 (1982).
41. Adams, J., Gerondakis, S., Webb, E., Corcoran, L. & Cory, S. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1982–1986 (1983).
42. Mushinski, J. F., Bauer, S., Potter, M. & Reddy, E. P. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1073–1077 (1983).
43. Kelly, K., Cochran, B., Stiles, C. & Leder, P. *Cell* **25,** 603–610 (1984).
44. Keath, E., Kelekar, A. & Cole, M. *Cell* **37,** 521–528 (1984).
45. Gonda, T., Sheiness, D. & Bishop, J. M. *Molec. cell. Biol.* **2,** 617–624 (1982).
46. Thomas, P. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5201–5205 (1980).

A novel serine esterase expressed by cytotoxic T lymphocytes

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Cytotoxic T (T_c) lymphocytes recognize and lyse target cells and are thought to serve as an important defence against viral infections[1] and possibly against neoplasms[2]. The nature of the receptors responsible for antigen recognition by these cells is becoming clearer, but the molecular mechanisms responsible for their cytolytic activity remain largely unknown. The possibility that proteases are involved in this process has been suggested by the effects of certain inhibitors[3,4]. Here we demonstrate that clones of murine T_c cells possess considerable trypsin-like esterase activity when assayed by a sensitive colorimetric assay. This activity was blocked completely by two serine esterase inhibitors, diisopropylfluorophosphoridate (DFP) and phenylmethylsulphonyl fluoride (PMSF), but not by N^{α}-tosyl lysyl chloromethyl ketone (TLCK). The use of ^{3}H-DFP as an affinity-labelling reagent demonstrated that the esterase activity resides in a protein of relative molecular mass (M_r) 28,000 (28K). A wide variety of other lymphocytes, including those from thymus, spleen and lymph node, established lines of B cells and noncytotoxic T cells, and clones of T helper cells, had about 300-fold less esterase activity than the T_c-cell clones and far smaller amounts of the DFP-reactive 28K protein. However, in thymocytes the esterase activity increased 20–50-fold and the 28K protein became more prominent 4 days after these cells had been stimulated *in vitro* to generate T_c cells.

Previous investigations have demonstrated that the lytic activity of both T_c cells[3,4] and natural killer cells[5–7] is reduced by exposure to certain protease inhibitors. In an attempt to characterize T_c-cell proteases directly, extracts of G4 cells, a clone of BALB.B anti-H-2D^d T_c cells[8], were prepared in phosphate-buffered saline (PBS) containing 0.5% Nonidet P-40 (NP40). Trypsin-like esterase activity in the lysate was assayed spectrophotometrically using a sensitive coupled reaction involving N^{α}-benzyloxycarbonyl-L-lysine thiobenzyl ester

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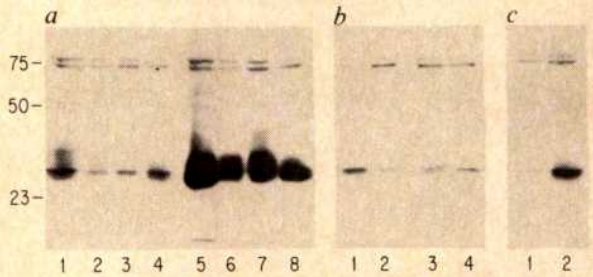


Fig. 1 Affinity labelling of serine esterase with ^{3}H-DFP. *a*, Affinity labelling of T_c cells. Three million T_c cells were labelled directly (lanes 1–4) or following solubilization in NP40-PBS (lanes 5–8). Lanes 1, 5, G4; lanes 2, 6, B10; lanes 3, 7, 1.5.2; lanes 4, 8, cr 15 (2 days exposure). *b*, Affinity labelling of T_h-cell extracts. Three million cell equivalents were analysed. Lane 1, O_3; lane 2, D_5; lane 3, A_6A_2; lane 4, BW5147 (3 days exposure). *c*, Affinity labelling of thymocyte extracts. Six million cell equivalents were analysed. Lane 1, resting thymocytes; lane 2, thymocytes incubated for 4 days in Con A supernatant and Con A (3 days exposure). M_r standards ($\times 10^{-3}$) are shown on the left.

Methods. [1,3-^{3}H]DFP in propylene glycol (5.2 Ci mmol^{-1}; NEN) was added to 300 μl of various cell extracts in NP40-PBS as described in Table 1 to a final concentration of 10^{-5} M. After 30 min incubation at 37 °C, the reaction was stopped by adding Tris-HCl *p*H 8.1, to a final concentration of 15 mM and SDS to a final concentration of 0.1%. Samples were centrifuged at 8,000*g* for 15 min. The pellets were discarded, the supernatants were concentrated to 100–150 μl and the labelled proteins recovered by cold acetone precipitation. The protein pellet was dissolved in sample buffer containing 5% mercaptoethanol and analysed by electrophoresis on a 10% polyacrylamide gel in the presence of 0.1% SDS (ref. 19). Gels were impregnated with Autofluor (National Diagnostics, Somerville, New Jersey), dried and exposed for the indicated times at −70 °C using XAR-5 film (Eastman Kodak).

Table 1 Esterase activity in extracts of cytotoxic T lymphocytes and other cells

Cells	Units per 10^6 cell equivalents	Cells	Units per 10^6 cell equivalents
Cytotoxic T-lymphocyte clones		Other	
1. G4	172-311	14. P815	0.518
2. G4 (IL-2)	203.4	15. Thymocytes	0.040
3. B10	67.4	16. Con A-activated thymocytes	3.47
4. 1.5.2	84.3	17. Resting spleen cells	0.557
5. cr 15	77.6	18. Splenic B cells	0.323
Noncytotoxic T lymphocytes		19. Splenic LPS blasts	0.136
6. O_3	0.520	20. B-cell clone 670.6	0.259
7. D_5	0.431	21. B-cell clone 670.11	0.251
8. A_6A_2	0.202	22. B-cell clone 750.17	0.227
9. EL-4	0.572	Inhibition studies	% Inhibition
10. EL-4 (RCASUP)	0.800	23. G4 (DFP-treated)	98.7
11. BW5147	0.258	24. G4 (PMSF-treated)	97.2
12. BW5147 (RCASUP)	0.268	25. G4 (TLCK-treated)	4.6
13. BW5147 (RCASUP+Con A)	0.365		

Cell preparations containing non-viable stimulator cells or complement-lysed cells were passed over Ficoll-Hypaque gradients (Pharmacia) to remove dead cells before extraction of the viable cells. G4 cells, which are adherent, were collected by brief exposure to EDTA (5 mM in PBS) after decanting stimulator cell debris; they were not purified further. Cells were washed twice in fresh medium, twice in PBS or in RPMI 1640 containing 100 $\mu g\ ml^{-1}$ bovine serum albumin (5 × recrystallized; Boehringer-Mannheim), counted, washed a third time in PBS, then lysed by incubating $1-2\times10^7$ cells ml^{-1} in PBS containing 0.5% NP40 (Particle Data Laboratories, Elmhurst, Illinois) for 20-30 min on ice with frequent vortexing. Esterase activity in the lysates was measured by adding 900 μl of a reaction mixture (0.2 M Tris-HCl *p*H 8.1, 2×10^{-4} M BLT (Calbiochem-Behring), 2.2×10^{-4} M dithiobis (nitrobenzoic acid); (Sigma)) to 100 μl of appropriate dilutions of cell extracts in NP40/PBS. After 30 min at room temperature, the absorbance at 412 nm was measured in a Gilford spectrophotometer using 100 μl 0.5% NP40 in PBS plus 900 μl of the reaction mixture as a blank; an absorbance of 1.0 was defined as 1 unit of esterase activity. The cell extracts were prepared from (1) G4, a BALB.B anti-H-2D^d T_c-cell clone (ref. 8); (2) G4 grown in recombinant human IL-2; (3) B10, a BALB.B anti-H-2L^d T_c-cell clone (ref. 8); (4) 1.5.2, a BALB.B anti-H-2L^d T_c-cell; (5) cr 15, a (B10.BR × B10.D2)F_1 anti-BALB minor T_c-cell clone (ref. 15); (6) O_3, a BALB/c T_H-cell clone specific for ovalbumin plus Ia^d (ref. 16); (7) D_5, a (BALB/c × A/J)F_1 T_H-cell clone specific for arsanilated protein plus Ia^d (ref. 17); (8) A_6A_2, a T_H-cell hybridoma of B10.A origin fused with BW5147, specific for hen egg lysozyme (provided by Dr L. Glimcher, manuscript in preparation); (9) EL-4, a T-cell leukaemia of C57BL/6 origin; (10) EL-4 maintained in 10% rat spleen cell Con A supernatant (RCASUP); (11) BW5147, T-cell lymphoma of AKR origin; (12) BW5147 maintained in 10% Con A supernatant; (13) BW5147 maintained in 10% Con A supernatant and 10 $\mu g\ ml^{-1}$ Con A; (14) P815, mastocytoma of DBA/2 origin; (15) thymocytes collected from 3-week-old BALB/c females; (16) thymocytes cultured for 4 days with 10% Con A supernatant and 10 $\mu g\ ml^{-1}$ Con A (ref. 10); (17) BALB/c spleen cells following lysis of erythrocytes with NH_4Cl; (18) BALB/c spleen cells depleted of T cells by treatment with two anti-Thy-1 monoclonal antibodies and rabbit complement; (19) BALB/c spleen cells cultured for 4 days with *Salmonella typhosa* lipopolysaccharide (LPS, Difco) at 10 $\mu g\ ml^{-1}$, then depleted of T cells with anti-Thy-1 before assay; (20-22) B-cell clones of CBA/N mice[18]. For the inhibition studies, an aliquot of G4 extract was incubated for 30 min at 37 °C with DMSO, or DMSO containing the indicated serine esterase inhibitors. Percentage inhibition = (1 − absorbance of treated samples/absorbance of control sample) × 100. (23) G4 extract pretreated with 2 mM DFP; (24) G4 extract pretreated with 2 mM PMSF; (25) G4 extract pretreated with 5×10^{-4} M TLCK.

(BLT) as described by Green and Shaw[9]; hydrolysis yields benzyl mercaptan, which reacts with Ellman's reagent to produce the thiophenoxide chromophore (Table 1). The esterase activity, equivalent to ~1 μg of trypsin per 10^6 cells, was not restricted to G4, as three other T_c-cell clones (B10, 1.5.2 and cr 15), of two different specificities, had roughly comparable activity (Table 1).

Because all of the T_c-cell clones had been maintained in long-term culture in medium containing 10% rat spleen cell concanavalin A (Con A) supernatant and stimulator cells, it was important to assay the stimulator cells and a variety of other cell lines. Table 1 summarizes the BLT-esterase activities in NP40 extracts of several different noncytotoxic cell lines. The cells used as stimulators to maintain the cultured T_c-cell clones (BALB/c spleen cells and *in vitro* passaged P815) possessed little activity. Two T-cell tumour lines, EL-4 and BW5147, also had minimal activity. Furthermore, when these T-cell lines were maintained in medium containing 10% Con A supernatant (or when BW5147 was incubated with both 10% Con A supernatant and 10 $\mu g\ ml^{-1}$ Con A) there was no significant increase in enzyme activity. Thus, the presence of stimulator cells and conditioned medium as sources of adsorbed esterase activity could not be invoked as trivial explanations.

Two additional experiments confirmed the endogenous character of the esterase activity in T_c cells. First, there was no difference between the esterase activity in G4 cells that had been serially cultured twice in recombinant human interleukin-2 (IL-2, a generous gift of Biogen) and in cells cultured continuously in 10% Con A supernatant. Second, and more interestingly, two T-helper (T_h) cell clones, O_3 and D_5, which lacked cytotoxic activity and had been maintained in long-term culture with 10% Con A supernatant and intermittently stimulated with antigen-pulsed spleen cells, had negligible activity (Table 1). A T_h-cell hybridoma, A_6A_2, also showed only traces of activity (Table 1). Thus, among all B and T lymphocytes tested, a high level of esterase activity was expressed only in T_c cells.

To determine whether the esterase activity in T_c-cell clones represented some unusual adaptation of these cells to long-term culture, we studied the expression of esterase activity in T_c cells generated by short-term culture of precursor cells. For this purpose, thymocytes were incubated in culture medium containing rat spleen cell Con A supernatant and Con A[10]. After 4 days, viable thymocytes showed a level of lectin-dependent cytotoxic activity (2-4 lytic units (LU_{50}, see Fig. 2 legend) per 10^6 cells)[11] that was comparable to that seen in conventional mixed lymphocyte cultures, and extracts of these thymocytes showed an approximately 50-fold increase in BLT-esterase activity (see Table 1).

The T_c-cell esterase activity was completely inhibited by the irreversible serine esterase inhibitors DFP and PMSF (Table 1), suggesting that this activity is due to a serine esterase, but it was not inhibited by TLCK, a trypsin inhibitor known to block T_c-cell cytotoxicity and to react with the surface glycoproteins T200 and LFA-1 (refs 3, 4, 8). Using ^{3}H-DFP as an affinity-labelling reagent[12] to characterize the esterase further (Fig. 1), we found that although minor ^{3}H-labelled components occurred at M_r 76,000 and 71,000, a prominent ^{3}H-DFP-reactive component was present at M_r 28,000. Moreover, whereas the higher-M_r moieties were present at about the same low level in all lymphoid cells tested, the level of the 28K component clearly

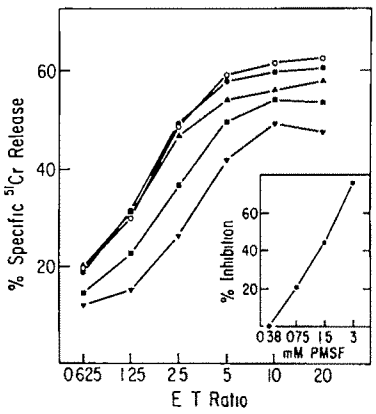


Fig. 2 Inhibition by PMSF of specific target cell lysis by T_c-cell clone G4. E:T, effector to target ratio. The G4 cells were suspended in complete medium at $\sim 5\times10^6$ ml^{-1} and DMSO (control) or PMSF dissolved in DMSO was added (final DMSO concentration 3%). ○, Control with DMSO alone, ●, 0.375 mM PMSF; ▲, 0 75 mM PMSF; ■, 1 5 mM PMSF; ▼, 3 mM PMSF. Inset: Results expressed as a dose-response curve. One lytic unit (LU_{50}) is the number of T_c cells required for 50% specific lysis of 1×10^4 ^{51}Cr-labelled P815 cells. Percentage inhibition = $(1-LU_{50}$ in the presence of PMSF/LU_{50} in the absence of PMSF) $\times 100$.
Methods. The cells were incubated with PMSF (or DMSO as control) for 30 min at 37 °C, washed, counted and diluted for titration of cytotoxic activity against 1×10^4 ^{51}Cr-labelled P815 cells. Recovery of G4 was comparable for all the treated samples. In some experiments, DMSO alone was responsible for ~10–15% ^{51}Cr release from labelled G4 cells, but the presence of PMSF at the concentrations shown had no additive toxicity measured either by ^{51}Cr release or by reduced recovery of viable (trypan blue-negative) G4 cells. T_c-cell assays were performed as described previously[20].

varied with the level of the BLT-esterase activity; both were pronounced in extracts of T_c-cell clones (Fig. 1*a*, lanes 5–8), but present in only trace amounts in noncytotoxic cells (Fig. 1*b*, lanes 1–4); and both increased after inducing T_c-cell activity in thymocytes by incubation with Con A (Fig. 1*c*, lanes 1, 2). Further evidence linking the BLT-esterase activity to the 28K component has emerged from their co-purification through affinity chromatography and an ion-exchange column (M. S. Pasternack *et al.*, manuscript in preparation).

If BLT-esterase is necessary for target cell lysis, inhibition of the esterase should block the cytolytic reaction. DFP was not tested because propylene glycol, the solvent in which it is kept, is toxic to cells, but PMSF at nontoxic concentrations clearly inhibited lysis of P815 (H-2^d) cells by the cloned anti-D^d G4 cells (Fig. 2). Note, however, that although PMSF at 2 mM blocked the esterase activity in lysates completely, at 1.5 mM it blocked only 50% of the T_c-cell cytolytic activity (Table 1, Fig. 2). Similarly, ^{3}H-DFP labelled the 28K component intensely in T_c-cell lysates, but only weakly in intact T_c cells (Fig. 1*a*, compare lanes 1–4 with 5–8). The difference between lysed and intact T_c cells raises several possibilities: one is that the BLT-esterase, or at least its active site, is normally located intracellularly, rather than on the cell surface, and is thus only poorly accessible to the added esterase inhibitors; another is that the esterase exists in intact cells as an inactive zymogen until converted into an active enzyme by another protein, perhaps a member of a complement-like cascade. In this connection, it is of interest that structures resembling the aggregated C9 component of complement have recently been described in cytolytic cells (T_c cells and natural killer cells)[13,14]. We believe the BLT-esterase described here represents the first enzymatic difference reported between cytotoxic and noncytotoxic T cells.

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Note added in proof: Preliminary results suggest that cloned T cells having both the helper phenotype (L3T4$^+$, Lyt-2$^-$, antigen-dependent IL-production) and exhibiting Con A-dependent cytotoxic activity may also have elevated serine esterase activity. Thus it is possible that the serine esterase level correlates with cytotoxic activity *per se*, rather than with the surface glycoprotein markets that classically distinguish helper (T_H) and cytotoxic (T_c) cells.

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1 Lukacher, A E, Braciale, V L & Braciale, T J *J exp Med* **160**, 814–824 (1984)
2 Engers, H D, Glasebrook, A L & Sorenson, G D *J exp Med* **156**, 1280–1285 (1982)
3 Chang, T-W & Eisen, H N *J Immun* **124**, 1028–1033 (1980)
4 Redelman, D & Hudig, D *J. Immun* **124**, 870–878 (1980)
5 Hudig, D, Haverty, T, Fulcher, C, Redelman, D & Mendelsohn, J *J Immun* **126**, 1569–1574 (1981)
6 Quan, P-C, Ishizaka, T & Bloom, B R *J Immun* **128**, 1786–1791 (1982)
7 Hudig, D, Redelman, D & Minning, L *J Immun* **133**, 2647–2654 (1984)
8 Pasternack, M S, Sitkovsky, M V & Eisen, H N *J Immun* **131**, 2477–2483 (1983)
9 Green, G & Shaw, E *Analyt Biochem* **93**, 223–226 (1975).
10 Irle, C, Piquet, P-F & Vassalli, P *J exp Med* **148**, 32–45 (1978)
11 Cerottini, J C & Brunner, K. *Adv Immun* **18**, 67–132 (1974).
12 Heck, L. W., Remold-O'Donnell, E & Remold, H G. *Biochem biophys Res Commun* **83**, 1576–1583 (1978).
13 Podack, E R & Konigsberg, P J *J exp Med* **160**, 695–710 (1984)
14 Henkart, P A, Millard, P J. Reynolds, C W & Henkart, M P *J exp Med* **160**, 75–93 (1984)
15. Hunig, T R & Bevan, M. J *J exp Med* **155**, 111–125 (1982)
16 Clayberger, C, DeKruyff, R H, Fay, R, Pavlakis, M & Cantor, H *J Immun* **133**, 1174–1178 (1984)
17 Rao, A, Faas, S J & Cantor, H *J exp Med* **159**, 479–494 (1984)
18 Braun, J. *J Immun* **130**, 2113–2116 (1983)
19 Laemmli, U K *Nature new Biol* **227**, 680–684 (1975)
20 Celis, E, Hale, A H, Russell, J H & Eisen, H N *J Immun* **122**, 954–958 (1979)

Reversible activation of non-steroid binding oestrogen receptor

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Two high-affinity oestrogen receptors have been identified in the chick oviduct with equilibrium dissociation constants (K_d) of 0.1 and 1 nM, differing in their binding kinetics, role in ovalbumin synthesis and independent regulation *in vivo*. The higher-affinity receptor (X) increases RNA polymerase II activity directly[1], whereas the low-affinity receptor (Y) seems to be necessary to confer specificity to transcription of oestrogen-dependent genes[2]. Acute administration of progesterone to oestrogen-stimulated chicks results in preferential destruction of the nuclear Y receptor[3] accompanied by interruption of ovalbumin gene transcription[4]. Here we demonstrate that receptor Y exists in a non-oestradiol binding form (Y_{nb}) which can be activated to the binding form *in vitro* by treatment with either ATP or ADP. Furthermore, dialysis of oviduct cytosol, which has no effect on the high-affinity receptor X, converts receptor Y to Y_{nb}; receptor Y can then be recovered by treatment with ATP in the presence of Mg^{2+} and independently of Ca^{2+}. This is the first report of the controlled interconversion between a non-steroid binding form of oestrogen receptor and active receptor in a tissue that contains two independently regulated oestrogen receptor types.

Oestrogen receptor augmentation by incubation with nucleotides has been reported by Fleming *et al.*[5] and Auricchio *et al*[6]. However, the affinity and steroid specificity of the induced binding sites were not examined. The characteristics of the heterogeneity of oestrogen receptors described in the chick oviduct[7–9] are different from the oestrogen-binding proteins of the rat uterus[10]. To avoid comparisons with the rat uterine types I and II oestrogen-binding proteins, the two oviduct receptors have been designated X ($K_d = 0.1$ nM) and Y ($K_d = 1$ nM).

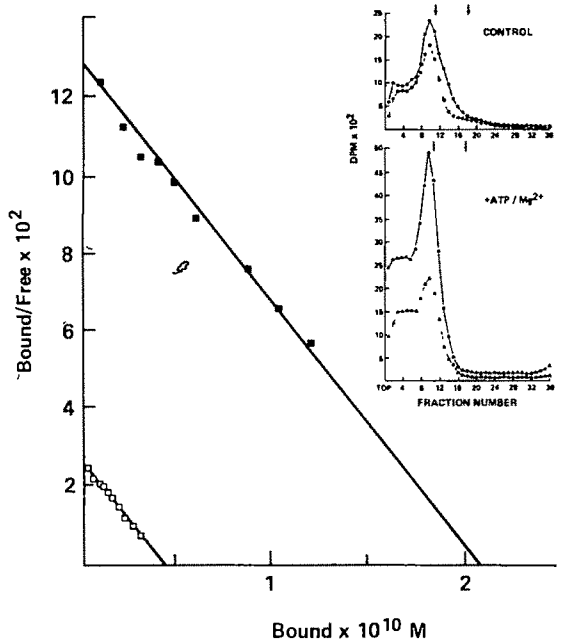


Fig. 1 Effects of ATP on the Y-receptor type. Week-old white Leghorn chicks were stimulated for 14 days with diethylstilboestrol in sesame oil and withdrawn for 1–2 days. Oviducts were removed into ice-cold saline and homogenized in TESH buffer (10 mM Tris, 1 mM EDTA, 12 mM thioglycerol, *p*H 7 4) in a ratio of 1/5 (w/v) and centrifuged at 1,000*g* for 20 min. Cytosol was obtained from the supernatant by centrifugation at 100,000*g* for 90 min. Binding assays used a range of ^{3}H-E_2 of 80 pM–10 nM and included 10 mM Na_2MoO_4 to stabilize the binding form of the receptor Nonspecific binding was assessed by addition of 100-fold excess diethylstilboestrol to duplicate tubes. Assays were incubated for 3 h at 30 °C, then for 18 h at 4 °C, unbound steroid was separated by addition of dextran-coated charcoal[4] and data analysed according to Scatchard[14] □—□, Control assay ($K_d = 1.5$ nM; $n = 0.43 \times 10^{-10}$ M), ■- - - -■, inclusion of 2.5 mM ATP and 2.5 mM $MgCl_2$ in the assay ($K_d = 1.6$ nM; $n = 2.0 \times 10^{-10}$ M). Insets Sucrose density gradient analysis. Aliquots of cytosol used in binding assays were labelled with 5 nM ^{3}H-oestradiol alone or with the addition of 2.5 mM ATP and 2.5 mM $MgCl_2$ at 30 °C for 3 h then at 4 °C for 18 h to augment receptor Y. Nonspecific binding was assessed by the inclusion of 100-fold molar excess unlabelled diethylstilboestrol. Unbound steroid was removed by the addition of dextran-coated charcoal immediately before the cytosol was applied to linear gradients containing 5–20% sucrose (w/v) with 400 mM KCl. Gradients were centrifuged for 9 h at 200,000*g* with ^{14}C-ovalbumin (3.6S) and ^{14}C-amylase (8.2S) included as internal standards Upper inset: Control; total bound (●- - - -●); nonspecific (○- - - -○). Lower inset: Augmented Y, total bound (▲—▲); nonspecific (△—△).

Oviduct cytosol, which contained low concentrations of receptor Y only, was obtained from chicks chronically stimulated with diethylstilboestrol for 14 days and then withdrawn for 1–2 days. Treatment of this cytosol with 2.5 mM ATP and 2.5 mM $MgCl_2$ in the presence of oestradiol at 30 °C for 3 h caused a large increase in the receptor concentration, as illustrated by Scatchard and sucrose density analyses (Fig. 1). This amplification was of a comparable degree when ADP was substituted for ATP, whereas GTP had a lesser effect. Both ATP with an ATP regenerating system (creatine kinase and creatine phosphate) and ADP with an ADP trap (creatine kinase and creatine) were effective. Incubation with the blocked ATP and ADP derivatives β,γ-methyleneadenosine 5′-triphosphate (AMP-PCP) and α,β-methyleneadenosine 5′-diphosphate (AMP-CP) did not increase the concentrations of Y, whereas α,β-methyleneadenosine 5′-triphosphate (AMP-CPP) was as potent as either ATP or ADP. Neither cyclic AMP nor cyclic GMP in the presence of isobutylmethylxanthine converted Y_{nb} to Y, nor did they alter the ATP effect. The conversion of Y_{nb} to Y by ATP was dependent specifically on Mg^{2+}, with an optimum concentration of 2–5 mM. Mn^{2+} was ineffective and, in contrast to the observations of Auricchio *et al.*[6], Ca^{2+} was ineffective and did not synergize the effect of Mg^{2+}. The augmentation did not occur in cytosol obtained from oviducts withdrawn from diethylstilboestrol for >3 days when a high concentration of X, as well as Y, was present. The data shown in Table 1 demonstrate that the augmented receptor has steroid specificity typical of oestrogen receptors.

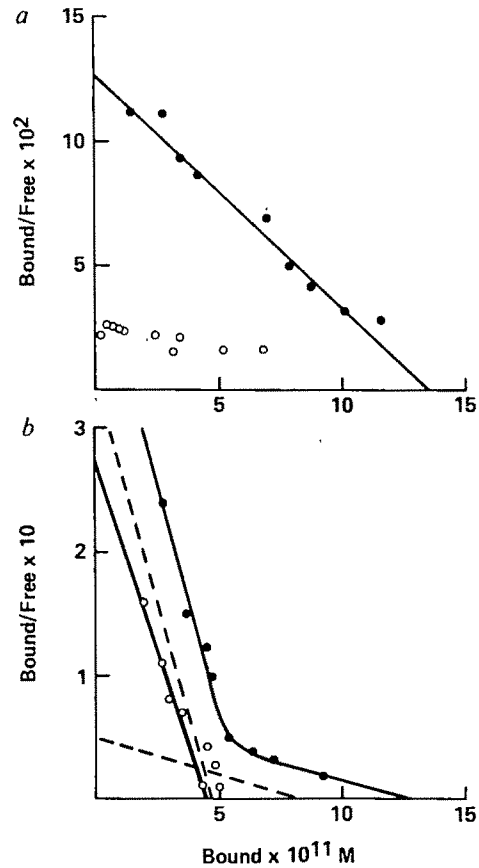


Fig. 2 Preferential effects of dialysis on the Y-receptor type. Chick oviduct cytosol was prepared, assayed and analysed as described in Fig 1 *a*, Oviduct cytosol from chicks withdrawn from diethylstilboestrol for 3 days containing the Y-receptor type only. Closed circles indicate control assay ($K_d = 1.1 \times 10^{-9}$ M, $n = 13.3 \times 10^{-11}$ M), open circles denote dialysis of cytosol for 24 h *b*, Oviduct cytosol from chicks withdrawn from diethylstilboestrol for 5 days containing both X- and Y-receptor types. Closed circles, control assay, dashed line, Rosenthal projection[15] of data; $K_dX = 0\,13 \times 10^{-9}$ M, $n = 4.3 \times 10^{-11}$; $K_dY = 1.5 \times 10^{-9}$ M, $n = 7\,9 \times 10^{-11}$ M; open circles, assay data from cytosol after dialysis for 24 h as in Fig. 1. $K_dX = 0\,13 \times 10^{-9}$ M, $n = 4.3 \times 10^{-11}$ M.

The Y-receptor concentration can be reduced selectively by dialysis (Fig. 2). Oviduct cytosol containing only the Y-receptor type was obtained from chicks withdrawn from diethylstilboestrol after 2 days. After this cytosol was dialysed for 24 h against TESH buffer (see Fig. 1 legend), oestrogen receptor was no longer detectable (Fig. 2*a*). Cytosol from chicks withdrawn from diethylstilboestrol for longer times (3–5 days) with both the X and Y receptors was also dialysed; no effect was observed on receptor X, whereas Y was decreased preferentially to undetectable levels (Fig. 2*b*).

Evidence for the reversible *in vitro* conversion of Y to Y_{nb} is presented in Table 2. Dialysis of cytosol caused a complete loss of the Y-receptor type. When dialysed cytosol was treated with ATP and $MgCl_2$, there was essentially quantitative recovery ($97.8 \pm 1.1\%$, $n = 3$) of the Y receptor. Treatment of dialysed cytosol with either $MgCl_2$ or ATP alone did not restore lost binding activity.

This study provides the first evidence that the chick oviduct oestrogen receptor Y exists in a non-oestradiol binding form, Y_{nb}, and that these two forms can interconvert; dialysis of cytosol favours Y_{nb}; Y_{nb} can be converted to Y at 30 °C in the

Table 1 Specificity of oestradiol binding to receptor Y after augmentation with ATP

Steroid (100 nM)	^{3}H-Oestradiol bound (pM) (% inhibition)
None	235 59 (0)
Oestradiol	124.77 (47.0)
Oestriol	163 43 (30.6)
Diethylstilboestrol	130.42 (44.7)
Progesterone	229.14 (2.7)
Testosterone	228.74 (2.9)
Dihydrotestosterone	227.79 (3.3)
Cortisol	236.06 (0)
Triamcinolone acetonide	237.82 (0)

Single-point (1 nM ^{3}H-oestradiol (E_2)) binding assays were performed in the presence of various unlabelled steroids in conditions of receptor augmentation as described in Fig 1 legend

presence of $MgCl_2$ and ATP, ADP or GTP. The Mg^{2+}-dependence of this conversion, the requirement of a hydrolysable terminal phosphate group for blocked nucleotide analogues to elicit conversion and the ineffectiveness of cyclic nucleotides (even with added ATP) suggest that a non-cyclic-nucleotide-mediated phosphorylation is involved. That both ATP and ADP, together with their regenerating and trap systems, respectively, augment receptor activity suggests that either ATP or ADP can donate the necessary phosphate group. The reversibility of this conversion (Table 2), the lack of receptor augmentation with the hydrolysis-resistant ATP and ADP derivatives and the effectiveness of ATP with regenerating system, eliminates the possibility that these observations are an ADP-mediated protection against heat loss, as has been suggested for glucocorticoid receptors[11].

We have been unable to observe the non-oestrogen binding form of the receptor in oviduct cytosol preparations from either chronically stimulated or long-term (>3 days) oestrogen-withdrawn chicks Because the highest concentration of Y_{nb} occurred when the cytoplasmic receptor concentration was lowest, it seems plausible that the non-binding form represents a recycling pool of receptor. It is also conceivable that Y_{nb} represents the first stage of irreversible receptor degradation. We do not favour the latter explanation for the following reasons: (1) the conversion of the non-binding to the oestrogen-binding form is possible simply by increasing the concentration of Mg^{2+} and specific nucleotides in the cytosol; (2) sucrose gradient analysis indicates that the receptor obtained by conversion from Y_{nb} has similar physical properties to the endogenous steroid-binding form; (3) although soluble receptor concentrations vary according to the time after withdrawal from diethylstilboestrol, the concentration of receptors after treatment with ATP/Mg^{2+} shows much less variation—after withdrawal from diethylstilboestrol, the concentration of Y_{nb} is inversely proportional to that of the oestradiol-binding receptors. Collectively, these data indicate that the controlled reversible interconversion between Y and Y_{nb} is associated with physiological receptor recycling. Furthermore, because recovery of Y receptors (which have been reduced *in vivo* or by dialysis treatment *in vitro*) can be accomplished by treatment of Y_{nb} with Mg^{2+} and ATP, the continued presence of ATP and Mg^{2+} may be necessary for maintenance of the steroid-binding characteristics of the Y form of the oestrogen receptor, similar to the mechanism proposed for the glucocorticoid receptor by Munck *et al.*[12]. The precise mechanism of these intriguing observations is currently under investigation.

Oestrogen receptor augmentation in the calf uterus, which contains only one form of active oestrogen receptor, has been shown recently by Migliaccio *et al.*[13] to be associated with a Ca^{2+}-stimulated phosphorylation. Although we agree that a phosphorylation reaction is associated with receptor augmentation, we do not find that Ca^{2+} stimulates this process in our system.

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Table 2 Reversibility of the conversion of oestrogen receptor Y to its non-oestradiol binding form

Treatment	Concentration of Y $\times 10^{10}$ M	K_d (nM)
Control	0.81	0.91
Dialysis	Undetectable	0
Dialysis, ATP/Mg^{2+}	0 78	1.0

Binding assays were performed on chick oviduct cytosol and analysed as described in Fig 1. Cytosol was dialysed against two changes of 1,000-fold excess TESH buffer for 24 h at 4 °C using Spectrapor dialysis tubing with a relative molecular mass cutoff of 3,500 ATP and Mg^{2+} were used in the presence of an ATP regenerating system according to the method of Barnett *et al*[11] Assays were incubated at 30 °C for 3 h then at 4 °C for 18 h. In three experiments, cytosol dialysis reduced Y concentration to undetectable levels, addition of ATP/Mg^{2+} resulted in recovery of 97.8±1.1% of Y.

1 Taylor, R N & Smith, R G *Biochemistry* **21**, 1791-1787 (1982)
2 Smith, R G & Taylor, R N *J Steroid Biochem.* **15**, 321-328 (1981)
3 McNaught, R W, Raymoure, W J & Smith, R G *Endocrinology* **112**, Suppl, 342 (1983)
4 Seaver, S S, Hoffman, J F & Coulson, P B *J Steroid Biochem.* **13**, 1269-1276 (1980)
5 Fleming, H, Blumenthal, R & Gurpide, E *Proc natn Acad Sci USA* **80**, 2486-2490 (1983)
6 Auricchio, F, Migliaccio, A, Castoria, G, Lastoria, S & Schiavone, E *Biochem biophys Res Commun* **101**, 1171-1178 (1981)
7 Smith, R G, Clarke, S G, Zalta, E & Taylor, R N *J Steroid Biochem* **10**, 31-35 (1979)
8 Taylor, R N & Smith, R G *Proc natn Acad Sci USA* **79**, 1742-1746 (1982)
9 Taylor, R N, Swaneck, G E & Smith, R G. *Biochem J* **192**, 385-393 (1980)
10 Clark, J H, Hardin, J W., Upchurch, S & Eriksson, H *J biol Chem* **253**, 7630-7634 (1978)
11 Barnett, C A, Palmour, R M, Litwack, G & Seegmiller, J E *Endocrinology* **112**, 2059-2068 (1983)
12 Munck, A *et al J Steroid Biochem.* **3**, 567-578 (1972)
13 Migliaccio, A, Rotondi, A & Auricchio, F *Proc natn Acad Sci USA* **81**, 5921-5925 (1984)
14 Scatchard, G *Ann NY Acad Sci* **51**, 660-672 (1949)
15 Rosenthal, H E *Analyt Biochem* **20**, 525-532 (1967)

Dihydropyridine receptors in muscle are voltage-dependent but most are not functional calcium channels

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1,4-Dihydropyridines are a new class of compounds believed to bind specifically and with high affinity to voltage-dependent calcium channels[1–5]. They may be the first example of a ligand of use in the extraction[1,2] and purification[3] of the Ca channel. Although Ca channels and dihydropyridine receptors are found in many tissues, the richest and most convenient source is skeletal muscle[4]. Functionally, 1,4-dihydropyridines such as nifedipine and nitrendipine block Ca channels[6–11]; this effect is believed to form the basis for their clinical importance as Ca antagonists in relaxing vascular smooth muscle[6]. But where currents through Ca channels can be measured directly[7–10], the block has required 100–1,000 times higher concentrations of dihydropyridine than necessary for the saturation of dihydropyridine binding sites[1,5]. This discrepancy has remained unresolved because the study of pharmacological effects on Ca channels has required intact cells, while it has been difficult to investigate binding in other than cell-free preparations. Here we describe a method for measuring dihydropyridine binding to intact skeletal muscle and we compare our results with voltage-clamp measurements of Ca-channel block. We conclude that less than a few per cent of the binding sites in skeletal muscle represent functional Ca channels, contrary to general belief.

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Figure 1*a* shows uptake of the 1,4-dihydropyridine ^{3}H-PN-200/110, a new and potent Ca antagonist[12,13] and close analogue of nitrendipine, by intact frog sartorius muscle While the drug was taken up, its concentration in the medium fell. The drug content of the muscles, calculated from the disappearance of drug from the medium, stabilized within 1-2 h Unfortunately, most of the uptake is an unsaturable distribution of the highly lipophilic drug into the hydrophobic reservoirs of the muscle To test for the presence of any saturable component of uptake, five pairs of frog sartorius muscles were incubated for 6 h in Ringer's solution containing ^{3}H-PN-200/110 at a final concentration of 0.6 nM With each pair, the first muscle was exposed to labelled drug alone and the second exposed also to a saturating concentration (700 nM) of unlabelled nitrendipine. After the muscles were blotted, weighed, digested and assayed for radioactivity, it appeared that nitrendipine reduced the uptake of the label ^{3}H-PN-200/110 by an average of 8.0± 0.8 pmol g^{-1}, indicating that the label and nitrendipine compete for a finite number of sites. However, a large and presumably nonsaturable uptake remained in nitrendipine (13.8 pmol g^{-1}) and made similar measurements at higher drug concentrations prohibitively noisy

To diminish noise, a protocol was developed to measure saturable and nonsaturable components on the same muscle. The experiment in Fig 1*b* is similar to that shown in *a*, but after equilibrium had been reached, a small volume of concentrated nitrendipine in ethanol was added to displace any saturably bound label (arrow). In the following 1-2 h, the muscle released label (1.9 pmol g^{-1}) and the concentration of ^{3}H-PN-200/110 in the medium increased from 1.6 to 2.1 nM (not shown). When ethanol is added without nitrendipine, release of the label is much less or does not occur

The experiment may be used to determine the amount of saturably bound label Before nitrendipine was added, the total uptake (B_t = 63 9 pmol g^{-1} in Fig. 1*b*) had saturable (B_s) and nonsaturable components ($B_{n,b}$), both in equilibrium with 1.6 nM label.

$$B_t = B_s + B_{n,b} \quad (1)$$

Two hours after adding nitrendipine, all label remaining in the muscle, $B_{n,a}$ (62.0 pmol g^{-1} in Fig 1*b*), should be contained in the nonsaturable pool. Subscripts 'b' and 'a' refer to values before and after addition of nitrendipine. $B_{n,a}$ should be larger than $B_{n,b}$ because the muscle is now in equilibrium with a higher concentration of label. If nonsaturable binding increases linearly with concentration, then

$$B_{n,b} = B_{n,a}[\text{label}]_b/[\text{label}]_a \quad (2)$$

In Fig. 1*b*, $B_{n,b} = 62.0 \times 1.6/2.1 = 47.2$ pmol g^{-1}; therefore, from equation (1), $B_s = 63.9 - 47\,2 = 16.7$ pmol g^{-1}. Thus, at 1 6 nM, the muscles in Fig. 1*b* bound 16.7 pmol g^{-1} of label that could be displaced by nitrendipine.

Figure 2*A* (curve *a*) was obtained from experiments similar to Fig. 1*b* and plots the amount of displaced label against the concentration measured just before the addition of nitrendipine. The curve describes one-to-one binding of label to a single class of sites. The parameters providing the best least-squares fit to the data are a dissociation constant (K_d) of 0.93 ± 0 07 nM and a binding capacity at infinite concentration (B_{max}) of 26 pmol g^{-1} (dashed line). Uptake was independent of whether ^{3}H-PN-200/110 was displaced by nitrendipine or by unlabelled PN-200/110 (five muscles). The filled circle was not included in the fit, it was obtained by counting muscles incubated with and without an excess of nitrendipine, as described above Within experimental error, the result agrees with the other data on curve *a*

When muscles were incubated in a potassium-rich solution (membrane potential −15 mV instead of the −88 mV observed in Ringer's), K_D for ^{3}H-PN-200/110 remained unchanged (1.00 ± 0.12 nM), but B_{max} was three times higher (79 pmol g^{-1}): The apparent increase in the number of binding sites cannot result from an increase in intracellular Ca^{2+} concentration

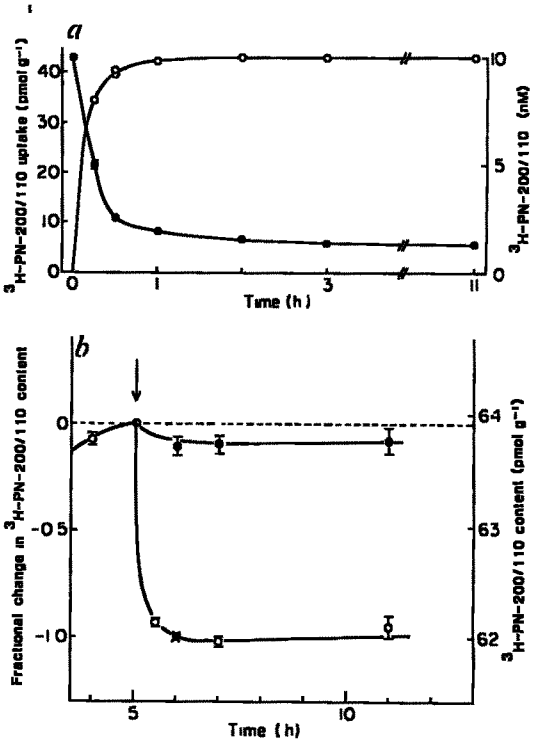


Fig. 1 *a*, Uptake of ^{3}H-PN-200/110 by intact frog sartorius muscles, *b*, release of ^{3}H-PN-200/110 by unlabelled nitrendipine. **Methods.** *a*, Each muscle (weight 40–110 mg) was incubated in a microcentrifuge tube containing 0.35 ml Ringer's solution (115 mM NaCl, 2 5 mM KCl, 1 8 mM $CaCl_2$, 3 mM phosphate buffer, *p*H 7 2), ^{3}H-PN-200/110 at an initial concentration of 10 nM and a 5-mm long magnetic stirrer for gentle agitation. At various times, 0 075-ml aliquots were withdrawn to measure the concentration (●) of label remaining in solution The drug content of each muscle was calculated from the disappearance of drug from the incubate, allowing for the fact that the volume was diminished by each sample. Drug content was normalized to that measured after a 1-h incubation, each point plotted (O) is the mean from five muscles In this experiment, the average uptake after 1 h was 43 ± 2 2 pmol per g wet weight; this defines the left ordinate. When no muscle was present, the concentration of label did not decline with time, indicating that no label was taken up by the microcentrifuge tubes. *b*, Protocol as in *a*, except that the first and second samples were always taken after 4 and 5 h of incubation, respectively After the second sample (arrow), 1 µl ethanol containing either no drug (●) or 1.5 mM nitrendipine (gift of Dr A Scriabine, Miles Institute, O, was added to the incubation tube and additional samples taken at later times. The added nitrendipine is expected to result, at equilibrium, in a concentration of ~700 nM, as the drug dilutes into an average nitrendipine space of 1 8 ml per muscle (30 5 ± 0.9 ml g^{-1}, n = 10, mean muscle weight 60 mg). This concentration is sufficient to displace all bound label, as the same amounts of label were released within 1 h of adding either 2 times smaller or 10-fold larger amounts of nitrendipine (not shown) The left-hand ordinate plots the change in muscle content of ^{3}H-PN-200/110 that occurred after the addition of ethanol and/or nitrendipine Throughout, the change in content was normalized to that at 6 h (O, ⊗). The average change was 1.9 ± 0 1 pmol g^{-1} (n = 20); this value, together with the average content at 5 h (63.9 pmol g^{-1} in this group of muscles), defines the right-hand ordinate ●, Not normalized Temperature, 22–24 °C throughout, ^{3}H-PN-200/110 (NEN, specific activity 85 Ci mmol^{-1}) was the optically pure (+) stereoisomer Vertical error bars indicate s e m and are omitted if smaller than the size of the symbol.

caused by release from the sarcoplasmic reticulum or, possibly, by failure of the Na/Ca exchange in the absence of external Na. First, incubation began long after the K-contracture accompanying the depolarization had subsided Second, the absence of Ca^{2+} inside and out had no effect on uptake (Table 1, experiment 1), confirming previous results on cell-free preparations[1] Apparently, depolarization acts like the drug diltiazem,

which, in cell-free[13] or even solubilized skeletal muscle preparations[2], also increases the number of nitrendipine or PN 200/110 binding sites without changing their affinity. The diltiazem result indicates that dihydropyridine receptors may exist in a form which does not bind antagonist dihydropyridines, or in which their affinity for them is too low to be detected by the usual assays. Diltiazem is thought to convert low- into high-affinity receptors; we suggest that depolarizarion has the same effect.

Figure 2*B* shows that the nonsaturable uptake depends linearly on concentration, as expected. Results in Ringer's and in high-K solution are identical. The regression line drawn through the data points corresponds to a PN-200/110 space of 27.5 ml per g wet weight.

The value for K_D obtained here agrees well with results from cell-free skeletal muscle preparations (for PN-200/110, K_D = 1.4 nM in guinea pigs[13]; for nitrendipine, K_D = 1.7 nM in rabbits[1]), as does the value for B_{max}. Because intact frog muscles have a protein content of 153 ± 10 mg per g wet weight (Lowry method, five muscles), B_{max} in depolarized muscle is 0.5 pmol per mg protein; in homogenized (and therefore depolarized) rabbit muscle, B_{max} = 0.8 pmol g^{-1} (ref. 1). This agreement supports the validity of our assay for dihydropyridine binding in intact muscle.

Table 1 shows that adrenaline (0.01 mM) causes no significant change in PN-200/110 binding (expt 2), even though the hormone increases current through Ca channels in cardiac muscle[14]. Muscles bound less ^{3}H-nitrendipine than ^{3}H-PN-200/110 (ref. 13), but the preference for PN-200/110 is apparently less marked in depolarized fibres (expts 3, 4).

Table 1 Uptake of dihydropyridines by frog sartorius muscles

Expt	Control	Test	Test/control
1	High K	Ca-free	1.15 ± 0.06
2	Ringer's	Adrenaline, 10 μM	0.91 ± 0.17
3	Ringer's	^{3}H-nitrendipine	0 22 ± 0.03
4	High K	^{3}H-nitrendipine	0.37 ± 0 02

Throughout, control and test conditions were compared on paired muscles (five muscle pairs per experiment) and specific uptake was measured as described in Fig 2 legend. Control: Incubation at final concentrations of 1.5-1 6 nM ^{3}H-PN-200/110 (Ringer's) or of 2.4-3 4 nM-(high-K solution; composition as in Fig. 2). Expt 1, the incubation fluid was Ca-free (40 mM tetraethylammonium (TEA^+)-EGTA, 65 mM TEA^+-aspartate, 5 mM TEA^+-MOPS, *p*H 7.0) and the muscles were chopped into 1-mm pieces to keep them depolarized and to allow this solution to enter the cytoplasm. Expt 2, as the control, but with 10 μM adrenaline added to the incubation fluid Expts 3, 4, as the control, but ^{3}H-nitrendipine (NEN) was used as label instead of ^{3}H-PN-200/110 The final concentrations of ^{3}H-nitrendipine (1.49 ± 0.06 nM in expt 3, and 3.37 ± 0.23 nM in expt 4) were larger than those of ^{3}H-PN-200/110, therefore, the uptake of ^{3}H-PN-200/110 was corrected (using K_D = 1.0 nM) to apply to the final concentrations obtained with ^{3}H-nitrendipine.

It is of interest to consider the number of binding sites observed here. With 80 pmol g^{-1}, there are four times more dihydropyridine sites than there are Na channels[15]; with a transverse tubule membrane area of 2,200 cm^2 per m of fibre volume[16], there are ~230 sites per μm^2 of that membrane. 80 pmol g^{-1} correspond to ~5×10^{13} sites per ml fibre volume, each representing a protein complex of relative molecular mass ~210,000 (ref. 17). This number of sites is large compared with the observed Ca current. At ~−20 mV, where Ca current is largest (Fig. 3*a*), its average value is 102 ± 9 mA ml^{-1} (n = 6). This corresponds to 2 fA per site, much less than the current through single Ca channels observed at similar potentials and external Ca^{2+} concentration (~0.1 pA in chromaffin cells[18] or 0.07 pA in cardiac myocytes[19]). Evidently, there are 35 to 50 times more dihydropyridine binding sites than there are voltage-dependent Ca channels that can be activated to pass current. This difficulty is not removed by considering that, at the normal negative resting potential, most dihydropyridine receptors have an undetectably low or no affinity for PN-200/110. Even recep-

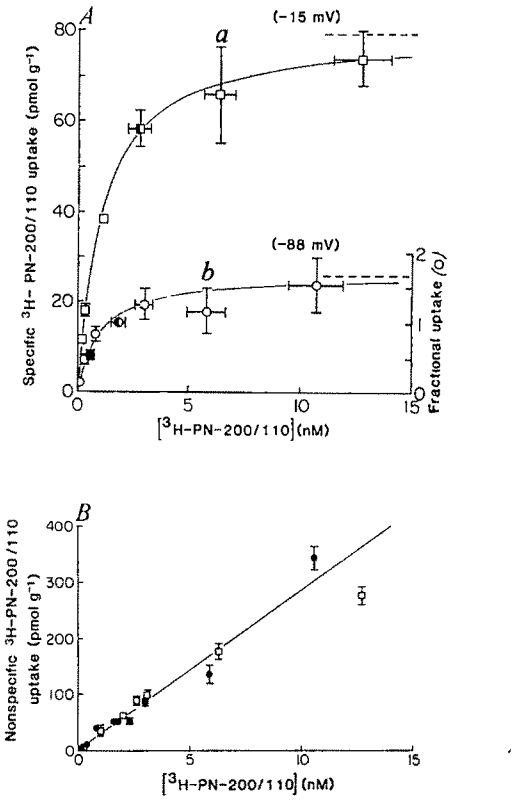


Fig. 2 *A*, Saturable uptake of ^{3}H-PN-200/110 determined from the drug contents and concentrations before and 2 h after adding an excess of unlabelled nitrendipine (see Fig. 1*b* and text) *B*, Non-saturable uptake against concentration in Ringer's (filled symbols) and high-K solution (squares).

Methods. Paired sartorius muscles were used; the first was incubated at an approximately fixed final reference concentration (1.9 nM in curve *a*; 2.7 nM in *b*), the second at various test concentrations. Specific uptakes (calculated from the displacement of label by nitrendipine) at the test concentrations were normalized to that at the reference concentration (the ordinate on the right of curve *a* is included as an example). Each data point represents measurements from at least five muscles; s.e.m. (uptakes) or s.d. (final concentrations) are indicated. Curve *a*, in Ringer's solution as in Fig. 1*b*, curve *b*, in a high-K solution with the same Ca^{2+} activity as Ringer's solution (40 mM K_2SO_4, 5 mM $CaSO_4$, 2 mM Tris-maleate, 113 M sucrose, *p*H 7.2). On first contact with the K-rich solution, muscles went through a transient contraction, during which time they were kept pinned out in a dish to prevent shortening. We rejected measurements from muscles that showed signs of contracture at the end of the experiment. To measure the membrane potentials in Ringer's, a muscle was incubated for 5 h and transferred to a dry recording chamber, then the incubate was poured over it. The average resting potential in this muscle was 87 5 ± 0.6 mV (n = 15). The membrane potential in the high-K solution is ~−15 mV. Curves *a* and *b* are least-squares fits of the function $B_s = B_{max} \times [label]/([label] + K_d)$. For least-squares fitting, uptakes and final concentrations were entered into the computer for each muscle, for the uptake, the ratio of test/reference was entered for the test muscle and a value of 1.0 for the reference muscle. Values for B_{max} giving the best least-squares fit (dashed lines) were 1.71 ± 0.04 times (Ringer's) or 1.35 ± 0.04 times (high-K solution) the reference uptake. Half-filled symbols represent uptakes (in pmol g^{-1}) at the reference concentrations; their values were 15 4 ± 0.7 pmol g^{-1} (n = 33) in Ringer's and 58 7 ± 3.8 pmol g^{-1} (n = 25) in the high-K solution These values define the ordinate on the left. At low concentrations, saturable and nonsaturable uptakes can be obtained more directly by comparing paired sartorius muscles incubated for 5 h in high-K solution One muscle was incubated without, and the contralateral muscle with, excess (700 nM) nitrendipine. Digesting the muscles and then counting radioactivity showed that, at 0.71-nM label, the uptake with nitrendipine was 24 4 ± 0.5 pmol g^{-1} less than that without (five muscle pairs). A group of five muscles from the same batch of frogs and processed as described in Fig. 1*b* took up 26.5 ± 4.1 pmol g^{-1} at 0.77 nM label, an identical result

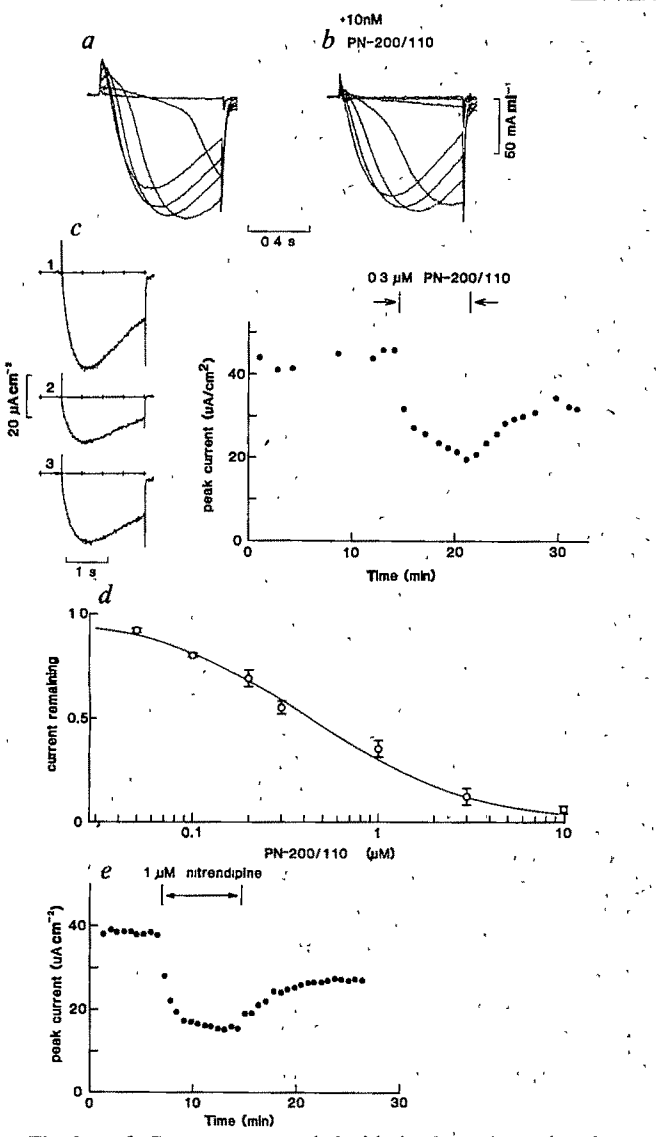


Fig. 3 *a*, *b*, Ca currents recorded with the three-electrode voltage clamp[22] from frog sartorius muscle fibres in a solution containing 107 mM TEA-methylsulphonate, 10 mM Ca-(methylsulphonate)$_2$, 10 mM MOPS buffer (*p*H 7.2) and 1 μM tetrodotoxin. ^{3}H-PN-200/110 binding in this solution was 1.40±0.22 times that in Ringer's solution (five muscle pairs). 350 mM sucrose was added to abolish contraction. Holding potential −90 mV; current traces were recorded during steps to between −60 or −50 mV and 0 mV in 10-mV increments. Ordinate calibration: At 23 °C in isotonic solution, the myoplasmic resistivity is calculated to be 150 Ω cm (ref. 23); adding 350 mM sucrose at that temperature increased the myoplasmic resistance per unit length 1.49±0.13-fold (n=7; microelectrode cable analysis). Consequently we used a myoplasmic resistivity of 223 Ω cm in equation (4) of ref. 22 to refer currents to the fibre volume in isotonic conditions. *a*, Control; *b*, from the contralateral muscle of the same animal after 6 h incubation in Ringer's +10 nM PN-200/110. The drug was retained continuously also after the incubation and during the recording period. *c*, Left, Ca-current during step depolarizations from the holding potential (−80 mV) to 10 mV before (1), during (2) and after (3) the presence of 300 nM PN-200/110. Right, peak current as a function of time, referred to the surface of a cylinder of the same length and diameter as the piece of fibre from which current was collected. External solution as in *a*, *b* except that sucrose was omitted. Vaseline-gap voltage clamp[7] with fibre ends cut in the solution of Table 1, expt 1. Throughout (*a*–*c*), currents were corrected for leakage and capacitive currents[7]. *d*, Peak current remaining 4–5 min after drug application as a fraction of that recorded immediately before; each point represents data from 3–8 experiments as in *c*. PN-200/110 (a gift of Dr C. E. Eden, Sandoz) was a 1/1 mixture of (+) and (−) stereoisomers. Their individual potencies are not known[12] but in the worst case one isomer would be inactive and the concentrations for the active isomer would be half those indicated. *e*, As *c*, but with nitrendipine.

tors in the low-affinity form are so abundant (79−26 = 53 pmol g^{-1} in Fig. 2) that at most a few per cent of them can represent functional Ca channels.

Figure 3*b* demonstrates that PN-200/110 has no or only small effects on Ca currents at concentrations high enough to load nearly all high-affinity sites. Although this muscle had been exposed continuously to 10 nM PN-200/110 for the previous 6 h, it retained Ca currents that were as large, or almost as large (87±5 mA ml^{-1}, n=7), as in the untreated contralateral muscle from the same animal (102 mA ml^{-1}). The slightly smaller average value with drug may result from the longer time that elapsed between dissection and recording (9–11 h). Hence, the drug can bind without blocking Ca channels, as has been suggested for synaptosomes[20].

At higher concentrations, PN-200/110 causes block, as do other 1,4-dihydropyridines. In Fig. 3*c*, 300 nM drug diminished Ca current to ~47% within 6 min, with partial recovery of current after drug removal. Other, similar experiments yielded the dose-response curve in Fig. 3*d*. The curve describes block by one-to-one binding of PN-200/110 to Ca channels (K_d ~430 nM). Prolonged application does not cause much stronger block. In other experiments (not shown), a semitendinosus muscle was incubated in 300 nM PN-200/110 for 5 h; then, with the drug still present, three single fibres were dissected over the next 4 h and currents recorded. Peak currents averaged 27±5 μA per cm^2 of fibre surface membrane. Four fibres from the drug-free contralateral muscle had previously given 58± 9 μA cm^{-2}. The difference is roughly consistent with Fig. 3*d*. Figure 3*e* shows block of Ca current by nitrendipine; this and two other such experiments suggest that ~700 nM of the drug is required to block half the channels.

Thus, PN-200/110 can bind without causing block, and there are many more high- (and low-) affinity binding sites than there are Ca channels. Both findings are explained most easily if most binding sites for antagonist 1,4-dihydropyridines are not functional Ca channels. In skeletal muscle, they may be unrelated to Ca channels, but this view is contrary to the circumstantial evidence in smooth and cardiac muscle and in phaeochromocytoma cells[21]. An alternative speculation is that dihydropyridine receptors represent a large reserve of dormant Ca channels kept for an unknown physiological emergency.

The voltage dependence of dihydropyridine binding described here may be compared with models proposed for cardiac muscle[11], where low- and high-affinity binding sites are thought to represent normal and inactivated Ca channels, respectively, that are in voltage-dependent equilibrium. In such a model, Ca channels should bind drug with a single apparent K_d (given in ref. 11) that diminishes in a graded fashion as the membrane potential is made more positive and the fraction of inactivated channels increases. The prediction is a competitive inhibition of drug binding by negative potentials, with a change of K_d but not of B_{max}. Figure 2 shows the opposite. In skeletal muscle, negative potentials evidently inhibit binding in a non-competitive fashion.

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1. Fosset, M., Jaimovitch, E., Delpont, E. & Lazdunski, M. *J biol. Chem.* **258**, 6086–6092 (1983).
2. Glossman, H & Ferry, D R. *Archs Pharmac.* **323**, 279–291 (1983).
3 Curtis, B M. & Catterall, W A. *Biochemistry* **23**, 2113–2118 (1984).
4 Gould, R J, Murphy, K M & Snyder, S H *Molec. Pharmac.* **25**, 235–241 (1984)
5. Bolger, G T. *et al. Biochem. biophys Res. Commun.* **104**, 1604–1609 (1982)
6 Fleckstein, A. *Calcium Antagonism in Heart and Smooth Muscle* (Wiley, New York, 1983)
7 Almers, W, Fink, R. & Palade, P. T. *J. Physiol., Lond.* **312**, 117–207 (1981)
8. Kass, R. S *J. Pharmac. exp Ther.* **223**, 446–456 (1982)
9 Lee, K S. & Tsien, R. W. *Nature* **302**, 790–794 (1983)
10 Almers, W & McCleskey, E W. *J Physiol., Lond* **353**, 586–608 (1984).
11. Bean, B P *Proc. natn. Acad Sci. U.S A.* **81**, 6388–6392 (1984).
12 Muller-Schweinitzer, E & Neumann, P J. *J cereb Blood Flow Metab* **3**, 356–361 (1983).
13. Ferry, D R., Goll, A & Glossmann, H. *Archs Pharmac.* **323**, 276–277 (1983)
14. Reuter, H. & Scholz, H *J Physiol., Lond.* **264**, 17–47 (1977)
15 Almers, W & Levinson, S R. *J. Physiol., Lond.* **247**, 483–509 (1975).

16 Mobley, B A & Eisenberg, B R *J gen Physiol.* **66**, 31-46 (1975)
17 Norman, R. I., Borsotto, M., Fosset, M & Lazdunski, M *Biochem biophys Res Commun* **111**, 878-883 (1983)
18 Fenwick, E M, Marty, A & Neher, E *J Physiol., Lond* **331**, 599-635 (1982)
19. Hess, P & Tsien, R W *Nature* **309**, 453-456 (1984)
20 Nachshen, D A & Blaustein, M P *Molec. Pharmac* **16**, 579-586 (1979)
21 Toll, L *J biol Chem* **257**, 13189-13192 (1982)
22 Adrian, R H, Chandler, W K & Hodgkin, A. L *J Physiol., Lond* **208**, 607-644 (1970)
23 Hodgkin, A L & Nakajima, S *J' Physiol, Lond* **221**, 105-120 (1972)

Central nervous system and peripheral nerve growth factor provide trophic support critical to mature sensory neuronal survival

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Primary sensory neurones in cranial and dorsal root ganglia (DRG) of adult animals are generally thought to be maintained through connections with their peripheral (but not central) targets by trophic factor(s) other than nerve growth factor (NGF)[1]. Damage to the peripheral process of sensory neurones results in a dramatic response or even death of the neurones, whereas axotomy (cutting) of the central process does not initiate profound reaction in these neurones. The development and maintenance of neurones are highly dependent on a supply of trophic agents produced by targets and retrogradely transported via the peripheral process to the cell body[2]. NGF deprivation in fetal rodents produced either by exogenously administered antibodies or by those of maternal origin, results in death of DRG[2-5] and of some cranial sensory neurones[6]. However, as chronic NGF deprivation in neonatal or adult rodents produces little or no cell death[7-10], it has been assumed that some other trophic factor(s) derived from the peripheral target sustains sensory neurones in postnatal life. By inducing NGF deprivation by autoimmunizing guinea pigs with mouse NGF and/or by cutting the central root (process) of a DRG, we demonstrate here that under certain conditions DRG neurones require NGF and centrally derived trophic support. Our results indicate that sensory neurones are maintained by the trophic support provided by both peripheral and central targets. This support is mediated by NGF and other as yet unidentified trophic factors. The relative importance of the two target fields and NGF compared with other trophic factors changes during development.

Chronic peripheral NGF deprivation was produced in young adult Hartley guinea pigs (Charles River) by immunizing with 2.5S NGF as described previously[4,8]. Age-matched control animals were not immunized. Two months after the immunization of the experimental group, surgical lesions to the central and/or peripheral processes of sensory neurones were performed as described in Fig. 1 legend. Three months after surgery, the effect of the single or combined lesions on neuronal number in L_6 DRG of nonimmunized control guinea pigs is shown in Table 1. Sciatic nerve crush injury results in a loss of ~20% of L_6 DRG neurones. As 80-85% of L_6 DRG neurones project down the sciatic nerve[8], 25% of the axotomized neurones die. In contrast, cutting of the dorsal root does not result in any cell loss, consistent with many reports and the data of Hinsey *et al.*[11], who found no decrease in neuronal number of S_2 lumbar ganglion in adult cat after dorsal root section. The data in Table 1 also show that combined lesions of the central and peripheral processes do not result in a statistically significant additional cell death, consistent with the observations of Hare and Hinsey[12] who observed that lesion of the central process did not increase the severity of the neuronal response to crushing of the sciatic nerve in cats killed 6-35 days after surgery.

The consensus that the central process has little or no role in providing trophic support of the sensory neurone clearly is not the case in immature animals. We have shown[13] in newborn rats that a loss of 50% of L_5 DRG neurones is produced by either a central or a peripheral lesion and that the combined lesion results in the loss of 70% of the neurones. Cell loss produced by either the single or combined lesion can be prevented by concomitant treatment with systemic NGF. Also, ^{125}I-labelled NGF can be efficiently and specifically retrogradely transported from the spinal cord to the DRG[13,14]. These experiments[13] demonstrate that the central process, as well as the peripheral process, has a critical role in providing trophic support to the developing sensory neurones in newborn rats and suggest that the trophic molecule provided by the central process is NGF.

Peripheral NGF deprivation by chronic exposure to anti-NGF in adults does not produce DRG neuronal death nor does it increase neuronal death in peripheral nerve crush[8]. It is clear, however, that even in adults, NGF deprivation has an adverse effect on DRG neurones. Chronic NGF deprivation in adults results in decreased levels of substance P[7] and a modest atrophy of neurones in DRG[8]. Thus, in contrast to the dependence of immature sensory neurones on peripheral NGF for survival, the mature neurone atrophies but does not die as a result of peripheral NGF deprivation. Similarly, the loss of trophic support from the central process results in cell death of immature DRG neurones; however, this loss of trophic support in adults is insufficient to cause cell death. We speculate that the conclusion that mature DRG neurones do not require NGF for survival may be incorrect because it ignores the possibility that

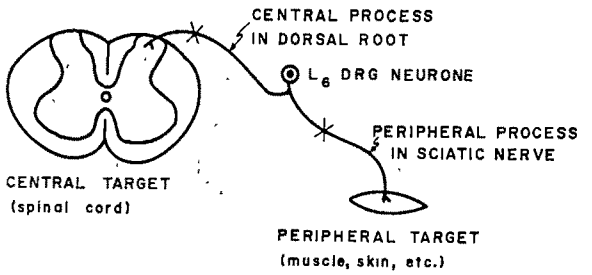


Fig. 1 Anatomy of the L_6 spinal sensory neurone showing the terminal areas of its central (spinal cord) and peripheral processes (for example, muscle). Crosses indicate lesion sites. Two months after immunization of the experimental group, animals were anaesthetized with chloral hydrate (350 mg per kg). Animals receiving a central lesion underwent an L_6 dorsal rhizotomy following exposure by a thoraco-lumbar laminectomy. The L_6 central process was sectioned near its entry to the spinal cord. Animals receiving a peripheral lesion underwent axotomy of their sciatic nerves. The nerve was exposed in midthigh and crushed for 40 s with a pair of jeweller's forceps at the tendon of the *obturator internus*. Animals were prepared with either a central or peripheral lesion alone or a combination of the lesions. Corresponding sham-operated control animals were prepared. Immunized animals were boosted with NGF monthly to maintain their titre of anti-NGF, and 90-100 days after lesion, they were perfused transcardially with saline and then with 10% formol saline. The sixth lumbar ganglia were removed bilaterally and immersed in 10% formalin and subsequently embedded in paraffin using standard histological techniques; 8-μm serial sections of the ganglia were stained with toluidine blue. The slides were coded so that their identity was unknown to the examiner. In every tenth section, neurones with a nucleolus were counted and the raw counts corrected[17].

Table 1 Neuronal numbers in L_6 DRG after lesioning of the central and/or peripheral processes in normal guinea pigs

Group	Lesion		Cell no. in L_6 DRG	% Control
	Central	Peripheral		
A	−	−	17,897 ± 711 (9)	100
B	+	−	17,439 ± 695 (4)	97
C	−	+	14,304 ± 1,184 (5)	79*
D	+	+	12,777 ± 847 (3)	71*

The data were analysed using two-way analysis of variance including Satterwaithe's correction of degrees of freedom resulting from unequal group size[16].

* Statistically different from control (group A); $P < 0.02$.

Table 2 Neuronal number in the L_6 DRG after lesioning of the central and/or peripheral processes in NGF-immunized guinea pigs

Group	Lesion Central	Lesion Peripheral	Cell no. in L_6 DRG	% Control
A	−	−	18,113±815 (7)	100*
B	+	−	13,589±996 (3)	75
C	−	+	14,695±726 (3)	81
D	+	+	10,641±711 (4)	59*

* Statistically different from all other groups (maximum $P < 0.054$)

NGF may be taken up by central terminals in the spinal cord and retrogradely transported by the central process to the DRG. Antibodies to NGF presumably would not cross the blood-brain barrier and would not prevent access of the DRG neurone to central nervous system (CNS)-derived NGF (or other trophic factor)

The data in Table 2 show the effects on sensory neurones of lesioning either the central or peripheral process or both in a group of animals chronically deprived of NGF. The peripheral lesion alone results in ~20% loss of DRG neurones[8]. However, in the NGF-deprived animal, cutting only the central process resulted in a 25% loss. In anti-NGF-producing animals subjected to a peripheral crush and a central lesion, a further neuronal loss of 27% (14,695-10,641) is observed. Thus, when the trophic support of peripherally derived NGF is removed, at least some populations of sensory neurones become dependent on centrally derived trophic support for survival. Conversely, when deprived of trophic support through the central process, at least a proportion of sensory neurones requires peripheral NGF for survival.

These results suggest that survival of sensory neurones in the adult animal depends on trophic support from both the central and peripheral targets. As peripheral NGF deprivation produces atrophy but not cell death of DRG neurones, whereas crushing the nerve does produce (~25%) cell death, we suggest that NGF is but one of possibly several peripherally provided molecules that trophically support DRG neurones. As NGF deprivation plus central lesion produces an amount of cell death comparable with that caused by peripheral crush lesion, the total trophic support provided by the central process is equivalent to the non-NGF component of peripheral trophic support. Thus, the loss of any one of these three trophic components (peripheral NGF, peripheral non-NGF, centrally derived factor) produces atrophy but not death. Removal of any two of these components results in loss of ~20% of cells. Loss of all three components would therefore be expected to result in the death of even more cells (Table 2). The data in Table 1 suggest that central process lesion may increase the severity of a peripheral crush in control animals, but the decrease in neuronal number is not statistically significant. Thus, we propose that the trophic support of a DRG neurone in the adult animal is represented by the sum of the trophic effects of NGF and other unknown factors derived from the periphery and a factor or factors derived from the CNS, one of which may be NGF. Based on our limited data, we cannot provide a complete explanation of this hypothesis. Interpretation is complicated by the fact that the DRG is composed of heterogeneous cell types. We do not know, for example, whether the 20% of cells that die after peripheral lesion are the same cells that die after peripheral NGF deprivation combined with a central lesion. In addition, the experiment used a peripheral crush lesion rather than a cut; thus, regeneration of the distal processes was possible and probably rather efficient[8]. Greater cell loss would be expected if the sciatic nerve were cut rather than crushed. Similarly, 15-20% of neurones in L_6 DRG do not project down the sciatic nerve and some neurones may reach the CNS via the ventral root, rather than the dorsal root[15].

Irrespective of the complications in providing a precise accounting of the cell death, the fact that trophic support for sensory neurones is provided via their central processes seems to explain the apparent lack of dependency of mature sensory neurones on NGF for survival, deduced by experiments using anti-NGF. The data suggest that survival of sensory neurones is dependent on relative trophic support provided by both the central and peripheral processes, although the quantitative support by the peripheral process is overall more important in adult animals. Because in newborn rats cell losses after lesioning of the central or peripheral process are the same[13], the results indicate that the relative importance of the trophic support provided by the central and peripheral processes changes as a function of age.

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1 Lieberman, A R *Int Rev Neurobiol* **14**, 49-124 (1971)
2 Hamburger, V & Oppenheim, R W *Neurosci Comment* **1**, 39-55 (1982)
3 Gorin, P D & Johnson, E M *Proc natn Acad Sci USA* **76**, 5382-5386 (1979)
4 Johnson, E M, Gorin, P D, Brandeis, L D & Pearson, J *Science* **210**, 916-918 (1980)
5 Aloe, L, Cozzani, C, Calissano, P & Levi-Montalcini, R *Nature* **291**, 413-415 (1981)
6 Pearson, J, Johnson, E M & Brandeis, L *Devl Biol* **96**, 32-36 (1983)
7 Schwartz, J P, Pearson, J & Johnson, E M *Brain Res* **244**, 378-381 (1982)
8 Rich, K M, Yip, H K, Osborne, P A, Schmidt, R E & Johnson, E M *J comp Neurol* **230**, 110-118 (1984)
9 Levi-Montalcini, R & Angeletti, P U *Physiol Rev* **48**, 534-569 (1968)
10 Thoenen, H & Barde, Y A *Physiol Rev* **60**, 1284-1335 (1980)
11 Hinsey, J C, Krupp, M A & Lhamon, W T *J comp Neurol* **67**, 205-214 (1937)
12 Hare, W K & Hinsey, J C *J comp Neurol* **73**, 489-502 (1940)
13 Yip, H K & Johnson, E M *Proc natn Acad Sci USA* **81**, 6245-6249 (1984)
14 Richardson, P M & Riopelle, R J *J Neurosci* **4**, 1683-1689 (1984)
15 Coggeshall, R E *Neurosurgery* **4**, 443-448 (1979)
16 Snedecor, G W in *Statistical Methods* (Iowa University Press, 1957)
17 Konigsmark, B E in *Contemporary Research Methods in Neuroanatomy* (eds Nauta, W H & Ebbesson, S O E) 315-340 (Springer, New York, 1970)

Erratum

Lower mantle heterogeneity, dynamic topography and the geoid

B. H. Hager, R. W. Clayton, M. A. Richards, R. P. Comer & A. M. Dziewonski
Nature **313**, 541-545 (1985).

IN this article Table 1 was omitted. It should read:

Table 1 Correlation coefficients for degrees 2-6 for model geoids predicted using two models of lower mantle heterogeneity (Dz and CC), convolved with dynamic response functions for Earth model U10

	Model geoid CC-U10					Model geoid Dz-U10				
Degree	2	3	4	5	6	2	3	4	5	6
Observed geoid	0.84	0.76	0.34	−0.17	−0.02	0.68	0.74	−0.24	0 24	0.17
Residual geoid	0.95	0.77	−0.25	−0.35	−0 33	0.82	0.79	−0.39	0.27	−0.03
Model geoid Dz-U10	0 79	0.66	0.01	0.12	0 31					

The models are correlated against the observed geoid, a residual geoid with slab effects removed[10], and each other

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Greek archaeomagnitudes

RELIABLE determination of the ancient geomagnetic field intensity is difficult; any new approach, such as that of Walton[1], is to be welcomed. Although the method is new and complex, it does not follow that it necessarily gives reliable answers, particularly as its validity rests on various theoretical assumptions about the behaviour of the magnetic carriers. We consider that Walton's dismissal of the linearity test in the commonly used Thellier technique is premature and insecurely founded. Certainly, this technique always needs to be carried out meticulously, as emphasized by Thellier, otherwise erroneous results are obtained that can, if taken at face value, lead to the supposition of rather exotic behaviour by the geomagnetic intensity in the past. Hence, Walton's finding that his earlier determinations using the Thellier technique were unreliable does not mean that all Thellier results are suspect.

In any linearity test the critical question is 'How linear is linear?' and in the examples referred to by Walton, it may well be that the criteria used were not sufficiently strict. On the other hand, we have confirmed the effectiveness of reliability criteria, where they are stringently applied, by re-examining our recent Thellier results[2] for the second millennium BC; we have remeasured several of our samples using the coercivity monitoring technique developed by Shaw[3] and find that the agreement is good. In this method, the coercivity spectrum of the natural remanent magnetization (NRM, the ancient magnetization) is compared with that of the thermoremanent magnetization (TRM, the magnetization acquired on cooling after the laboratory heating); changes in shape indicate that the laboratory heating has caused mineral alteration and there is risk of error. Also, the coercivity spectrum of an acquired remanent magnetization (ARM, magnetization acquired from a weak steady field in the presence of a strong alternating field) given before heating is compared with that of an ARM given after heating; as well as spectrum shape, this monitors any increase or decrease in the magnetic carriers in each coercivity interval, thus providing a built-in quantitative measure of the magnetic alteration. In the basic Shaw technique the ancient intensity is obtained from the NRM/TRM ratio evaluated from coercivity intervals for which there is no change in ARM strength. In later developments[4,5] it has been shown that, even if alteration occurs, the ARM ratio can be used to correct the NRM/TRM ratio, at least for coercivity intervals >100 mT.

We have now used the Shaw technique, or one of its variants, on eleven samples from several of the sites in ref. 2 (see Table 1). There is agreement with the Thellier values to within 15% for nine of the samples; seven of these are within 10% with the Shaw values on average 5% lower than the Thellier values. The two remaining samples gave inconsistent results for different coercivity intervals, although always within ±25% of the Thellier value. These are our first results using the Shaw technique and, because there is scope for improvement in our facilities, we consider that good confirmation of the validity of the Thellier results has been obtained. Also, we have used the ARM technique on subsidiary cores (19 samples), with intervening heatings to various of the temperature steps used in the Thellier determination. In this way we can determine whether or not alteration has set in by the upper temperature limit of accepted Thellier data; in no case has the ARM change at that limit been >6%. These tests justify our confidence in the results of ref. 2.

Finally, regarding the alteration test of comparison between two measurements made in zero field, suggested by Walton for the Thellier method, we note that his procedure monitors only those carriers having blocking temperatures greater than the magnetization temperature; on the other hand, the in-field measurement is also affected by carriers having lower blocking temperatures. Our experience of comparing demagnetization curves before and after heating indicates that alteration can occur in some blocking temperature ranges without affecting others. A much better test is to compare two in-field measurements, but tests of this type can never give conclusive proof that alteration has not occurred because of the probability that it occurs rapidly and is effectively complete during the first stage of a given temperature step. These comments apply equally to the Walton method.

Table 1 Comparison of Shaw and Thellier techniques

Presumed date BC	Sample location	Intensity ratio (Thellier)	Ancient intensity (μT) Thellier	Shaw
1550–1500	Apheq, Israel	1.06	45	43
1550–1500	Apheq, Israel	1.15	49	48
1504–1492	Karnak, Egypt	1.17	45	40
1250–1150	Karnak, Egypt	1 51	58	57
1250–1150	Karnak, Egypt	1 53	59	57
1150–1075	Kition, Cyprus	1 62	72	71
1100–950	Karnak, Egypt	1.52	59	51
1100–950	Karnak, Egypt	1.52	59	61
1050–1000	Koukhia, Cyprus	1.53	68	68

The Thellier results are from ref. 2; the intensity ratio quoted is F_A/F_D, where F_A is the ancient intensity and F_D is the intensity at the site attributable to an axial centred geomagnetic dipole of present-day strength (8×10^{22} A m^2)

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1. Walton, D *Nature* **310**, 740–743 (1984)
2. Aitken, M J, Allsop, A L, Bussell, G D & Winter, M B *Nature* **310**, 305–306 (1984)
3. Shaw, J *Geophys J R astr Soc* **39**, 133–141 (1974)
4. Kono, M *Geophys J R astr Soc* **54**, 241–261 (1978)
5. Rolph, T C & Shaw, J *Geophys J R astr Soc* **80**, 773–781 (1985)

WALTON REPLIES—There is quite a clear disagreement between recent results for Greece[1] and those of Aitken *et al.*[2].

I have published two sets of archaeomagnetic intensity measurements for Greece. The original data[3] also show the increase in intensity that Aitken *et al.* are proposing to use for archaeomagnetic dating. Unfortunately, the old data in ref. 3 are wrong, which prompted the study in ref. 1. This later study showed that the error was caused by mineral alteration at the higher measurement temperatures. A few samples that showed no evidence of alteration did not show an increase in intensity. It was possible to correct for a small degree of alteration; if the effects of alteration were retained, the increase in intensity referred to above was present in the high temperature points but not at low temperatures. On correction, all the points at all temperatures were consistent, within the experimental accuracy, and the increase disappeared.

The experiments reported in ref. 3 used a technique that did not differ substantially from that used by Aitken *et al.*[2], but Aitken *et al.*[2] express reservations about the technique used in ref. 1. It is important, however, to recognize that in all three methods the ancient intensity is obtained by the same method, thermal demagnetization and magnetization, differing only in the test used for alteration.

More specifically, the method used by Aitken *et al.*[2], and initially by myself,

method 1, compares the moment lost after demagnetizing for a fixed time at a given temperature, with the moment gained in a known field after holding for the same time at the same temperature. My new method, method 2 (see ref 1), also compares the moment lost in zero field on holding the sample for a fixed time at a temperature with the moment gained in the same time period in the laboratory field. In method 2, because the moment of the sample is being monitored continuously, several readings can be made while the sample is hot and, to improve the statistical accuracy, the slope of the best straight line through the points is used instead of simply taking the difference between the first and last reading Thus, the 'theoretical assumptions' on which our methods depend are, up to this point, identical.

The two methods do differ in the means they use to detect alteration, as discussed in refs 1,2. The important point is that alteration was detected using method 2, but was not detected using the linearity test of ref. 2

In the event that alteration is complete by the end of the first heating in method 2, it would still be detected as a sudden change in the value of the ancient intensity at that temperature. Method 2, in other words, also incorporates a 'linearity test'

In any case, the point is not that alteration has been missed but that it has been detected. Almost all the material between 1200 and 300 BC showed evidence of alteration at temperatures below 350 °C. If the altered points were used, high values were obtained for the ancient field in good agreement with refs 2 and 3 If points with no evidence of alteration were used, the increase that Aitken *et al* report, forming the basis for their dating method, disappears.

It will be useful to compare these results with those obtained using the Shaw method. At present Aitken *et al.* report nine values. Of these, three are dated 1500 BC where there is no disagreement. In assessing the remaining six it is important to consider their internal consistency. results between 1250 and 950 BC are between 58 and 72 μT with the Thellier and 51 and 71 μT with the Shaw method. In the same period, nine lie between 46 and 54 μT With so few results the difference is hardly significant

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1 Walton, D *Nature* 277, 643–644 (1979)
2 Aitken, M J, Allsop, A L, Bussell, G D & Winter, M *Nature* 310, 306–308 (1984)
3 Walton, D *Nature* 310, 740–743 (1984)

A Grenville Sm/Nd age for the Glenelg eclogite

SANDERS *et al*[1] present data indicating that eclogites within the eastern Lewisian inlier at Glenelg equilibrated at ~1,100 Myr BP at temperatures and pressures of ~700 °C and >12 kbar. On the basis that the overlying Moine cover shows no sign of having exceeded conditions for the lower amphibolite facies, they conclude that the Moine was deposited unconformably on a deeply eroded Lewisian basement following Grenville age metamorphism, a view at variance with much recent work which regards the Moine as a series of late mid-Proterozoic metasediments that underwent widespread metamorphism and deformation at ~1,100–1,000 Myr BP[2–5] Whilst the data produced by Sanders *et al*[1] is not in dispute, their conclusions relating to the conditions of metamorphism of the eclogites and thus the age of sedimentation of the Moine may be questioned.

We consider the suggestion of Sanders *et al.*[1] that the eclogites were metamorphosed at pressures of >12 kbar questionable on four counts. (1) Mineral parageneses and tabulated phase chemistries are not those of an eclogite in the strict sense but of a medium- or high-pressure granulite. Consequently, deriving a pressure by analogy with Green and Ringwood's[6] eclogite field is inadvisable. More realistic pressures of as low as 6 or 7 kbar could be derived from their granulite field. (2) Although Sanders[7] implies that he has corroborative evidence from the garnet/plagioclase/kyanite/quartz geobarometer, this is as yet unpublished and, in view of the problems inherent in modelling plagioclase and garnet activities, may not be conclusive. (3) We have examined staurolite/garnet/kyanite/biotite/quartz-bearing gneisses from the eastern Lewisian in which, on textural criteria, we are confident that all coexisting phases are of Grenville rather than Caledonian age. At temperatures of 650–700 °C, staurolite and quartz will not be stable at pressures in excess of 8 kbar[8,9]. (4) It has been repeatedly shown that garnet-pyroxenite assemblages comparable to those described from Glenelg are stable at relatively low pressures in rocks undergoing dry metamorphism at amphibolite facies conditions[10–12] These pressures could well be consistent with both the pressure stability limits of staurolite and quartz and pressure estimates of 6–8 kbar obtained from mineral assemblages coeval with the early pre-Caledonian metamorphism within the Morar division Moine (D. Barr, personal communication), in which case there may be no significant difference between the metamorphic conditions of the Eastern Lewisian and those of the Moine

In this light a more reasonable explanation is that the ~1,100 Myr BP age[1] dates both the equilibration of the eclogites and the initial amphibolite facies metamorphism of the Moine. Isotope data considered to relate directly to the age of initial metamorphism of the Moine includes Rb/Sr whole-rock ages of ~1,100–1,000 Myr BP on the Morar and Lochailort pelites and the Ardgour gneiss[13–15], which are interpreted by Sanders *et al.*[1] as dating sedimentation and diagenesis rather than metamorphism Whilst the interpretation of the isotope data relating to the metasediments is equivocal[16], we note that the Ardgour gneiss is now generally regarded as a member of an extensive suite of deformed granitic intrusions where emplacement was coeval with amphibolite-facies metamorphism and widespread deformation within the Moine[3–5,17]. Given that there is no indication of any major structural or metamorphic break of Precambrian age between the south-west Moine and Glenelg[18], it seems difficult to avoid the conclusion that the initial metamorphism of the Moine occurred at ~1,100–1,000 Myr BP.

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1 Sanders, I S, van Calsteren, P W C & Hawkesworth, C J *Nature* 312, 439–440 (1984)
2 Powell, D, Baird, A W, Charnley, N R & Jordan, P J *J geol Soc. Lond* 138, 661–673 (1981)
3 Roberts, A M & Harris, A L *J geol Soc Lond.* 140, 883–892 (1983)
4 Harris, A L in *Geology of Scotland* (ed Craig, G Y) 1–22 (Scottish Academic Press, Edinburgh, 1983)
5 Strachan, R A *Scott J Geol.* (in the press)
6 Green, D H & Ringwood, A E *Geochem cosmochim Acta* 31, 767–833 (1967)
7 Sanders, I S *Spec Rep geol Soc Lond* 8, 97–100 (1979)
8 Ganguly, J *J Petrol* 13, 335–365 (1972)
9 Yardley, B W D *Neues Jb Miner Mh*, 127–132 (1981)
10 Turner, F J *Metamorphic Petrology* p 524 (McGraw-Hill, New York, 1980)
11 Bryhni, I, Bollingberg, H J & Graff, P R *Norsk geol Tidsskr* 49, 133–225 (1969)
12 De Wit, M J & Strong, D F *J Geol* 83, 609–627 (1975)
13 Brook, M, Powell, D & Brewer, M S *Nature* 260, 515–517 (1976)
14 Brook, M, Powell, D & Brewer, M S *J geol Soc. Lond* 133, 489–496 (1977)
15 Brewer, M S, Brook, M & Powell, D *Spec Rep geol Soc. Lond* 8, 129–137 (1979)
16 Van Breemen, O, Halliday, A N, Johnson, M R W & Bowes, D R *Geol J spec Issue* 10, 81–106 (1978)
17 Barr, D, Roberts, A M, Highton, A J, Parson, L M & Harris, A L *J geol Soc. Lond* (in the press)
18 Powell, D *J geol Soc. Lond* 130, 575–593 (1974)

SANDERS REPLIES—Strachan and Treloar favour contemporaneous (1,100–1,000 Myr BP) metamorphism for both the eclogite-bearing basement and the Moine cover in the Glenelg region. To sustain their view, they suggest that the ab normally high metamorphic pressure

(>12 kbar) inferred for the basement[1] is a serious overestimate.

Although published evidence relating to metamorphic pressure/temperature (P/T) conditions for the Glenelg eclogite may not be extensive, I contend that the rock and mineral analyses reported (Table 1, ref. 1) are more than sufficient to demonstrate a high-pressure origin. The two eclogite samples have tholeiitic chemistry and are plagioclase free, they are therefore true eclogites (rather than high-pressure granulites) and reference to Green and Ringwood's[2] eclogite field is therefore valid Moreover, omphacite coexists with quartz in S274 and the mole fraction of jadeite in the omphacite is 0.31 (calculated as Al(VI) minus Al(IV) in a 4-cation formula). Assuming a disordered pyroxene structure, the omphacite composition implies a pressure >15 kbar at 700°C (ref. 3). The temperature estimate of 700°C is, incidentally, supported by a large body of unpublished analytical data relating to garnet/clinopyroxene thermometry and calcite solvus thermometry

Against this P/T background, Strachan and Treloar's staurolite/quartz-bearing assemblage from the Glenelg basement may best be regarded as a product of the widespread retrogressive metamorphism that has affected the eclogite and other high-grade rocks at Glenelg[4] According to Strachan and Treloar, the staurolite/quartz-bearing assemblage is equivalent in terms of P/T conditions to the Moine Thus, it would seem that retrogression in the basement correlates with prograde metamorphism in the cover, which is, of course, consistent with the view that the Moines were deposited on an eroded Grenville basement. With regard to the Ardgour gneiss, I would argue that the case for intrusion at about 1,000 Myr BP is considerably weakened by the real possibility that the gneiss is a metasediment with a derived Rb/Sr isotope system.

In my view, any claim that the Moines have a Grenville metamorphic history carries with it the implication that they underwent early eclogite-facies metamorphism, at least in the Glenelg area. Hitherto, evidence for such an event has not been reported

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1 Sanders, I S, van Calsteren, P W C & Hawkesworth, C J *Nature* 312, 439-440 (1984)
2 Green, D H & Ringwood, A E *Geochim cosmochim. Acta* 31, 767-833 (1967)
3 Holland, T J B *Contrib Miner Petrol* 82, 214-220 (1983)
4 Ramsay, J G *Q Jl geol Soc. Lond* 11, 487-523 (1958)

Proper solar wind power estimation and planetary radiometric efficiencies

DESCH and Kaiser[1] have computed the average absolute efficiencies of the radio planets for electromagnetic wave production, restricting their attention to the emissions known to be controlled by solar wind energy inputs to the magnetospheres of the Earth, Jupiter and Saturn. Our purpose here is to offer a word of caution about the methods, assumptions and conclusions of their paper and to suggest that radio emissions for unexplored planets, such as Uranus, may be much different from what they determined.

Their method[1] was to compute the solar wind input power, P_i, to the various magnetospheres by estimating the solar wind kinetic energy flux (ρV^3) at the planetary location and to use the square of the Chapman–Ferraro length ($l_0 = (M^2/4\pi\rho V^2)^{1/6}$) as an estimate of the effective energy 'collection' area of the magnetosphere Here M is the planetary magnetic moment, ρ is the solar wind mass density (g cm^{-3}) and V is the solar wind speed, so that ρV^3 has units of erg cm^{-2} s^{-1} Then, using estimates of the planetary radio emission power, P_r (at a standard distance of 1 AU), the authors computed an efficiency, from the ratio P_r/P_i

The fault with this method is demonstrated in Fig. 1 Visual inspection of Fig. 1 shows that the AE index correlates quite well with the ε, or P_{VB_s}, parameter (correlation coefficient ~0.7), but the time profile of AE bears little specific relationship to the P_k parameter Detailed study has shown conclusively[2-4] that ρV^3, in the case of the Earth, is not the solar wind parameter that 'drives' auroral disturbances. The results shown in Fig 1 are quite general As AE is a measure of auroral activity and can be used[3] to estimate auroral particle precipitation and auroral Joule heating, we may conclude with confidence that P_k does not correlate well with these primary forms of terrestrial auroral energy dissipation Furthermore, as it is well known[5] and indeed implicit in the argument of Desch and Kaiser, that auroral kilometric radiation (AKR) is well correlated with AE, we may also conclude that P_k is not well correlated with AKR.

The reasoning is thus as follows. The quantity ρV^3 is poorly correlated (on substorm timescales) with VB^2 in the solar wind, but it is VB^2 (or a refinement thereof) which controls substorm (AE) activity at Earth Because it is clearly substorm activity, and collective plasma processes during substorms, that control the generation of AKR, solar wind estimates of ρV^3 are an improper estimate of P_i as a causal agent of AKR (or P_r), in the case of the Earth. By extension, the computed radiometric efficiency estimates[1] must be regarded as being predicated on erroneous assumptions.

Now Gallagher and D'Angelo have shown a very strong dependence of AKR on the solar wind speed only[6], but as they used very long-term averages, their results were strongly time aliased. Indeed, if one looks at long-term averages, geomagnetic activity is well correlated with the solar wind speed[4,7], but this says almost nothing about the solar wind–magnetosphere coupling mechanism or substorm energy source as substorms occur on timescales of $\lesssim$ 1 h.

The criticism raised here might be less significant if ρV^3 were of a similar magnitude as $VB^2/8\pi$, but it is almost always true that ρV^3 is ~100 times larger than $VB^2/8\pi$. This is well illustrated in Fig 1. Thus, as it is the electrostatic[4] or electromagnetic (Poynting)[3] energy in the solar wind which drives auroral activity, the values of efficiency derived by Desch and Kaiser should be ~100 times greater in the case of the Earth[8].

We really do not know how similar or dissimilar the generation of Saturn kilometric radiation (SKR) is to AKR Furthermore, we are uncertain of the generation mechanism of the hectometre-wavelength (HOM) emission of Jupiter. A further exacerbating feature is that we have little clear evidence as to whether solar wind–magnetosphere coupling proceeds by the same physical processes at Saturn and Jupiter as it does at the Earth If precisely the same processes were to occur (that is, dayside magnetic reconnection and magnetotail substorm initiation[8]), we would estimate that the SKR and HOM efficiencies should also be ~100 times greater than estimated by Desch and Kaiser. On the other hand, if solar wind kinetic energy flux variation were driving the HOM and/or SKR components, then the values of efficiency would be $\sim 5 \times 10^{-4}$ for the Earth, but would be only $\sim 5 \times 10^{-6}$ for Jupiter or Saturn (as estimated by Desch and Kaiser).

In conclusion, based on a proper computation of the energy entering the Earth's magnetosphere, we conclude that terrestrial AKR is produced with a much higher efficiency than computed by Desch and Kaiser. Similarly high efficiencies may or may not apply in the cases of Jupiter and Saturn, this depends very strongly on the physical processes responsible for solar wind–magnetosphere coupling in those cases Because of such uncertainties, the radiometric estimates made for Uranus[1] must be regarded with caution. In view of the 'pole-on' interaction of Uranus with the solar wind mentioned by Desch and Kaiser, the nature of solar wind coupling may be very different for any possible Uranus magnetosphere. At the very least, readers should appreciate that in the Earth's magnetosphere it is electromagnetic, not particle, energy in the solar wind that drives geomagnetic activity.

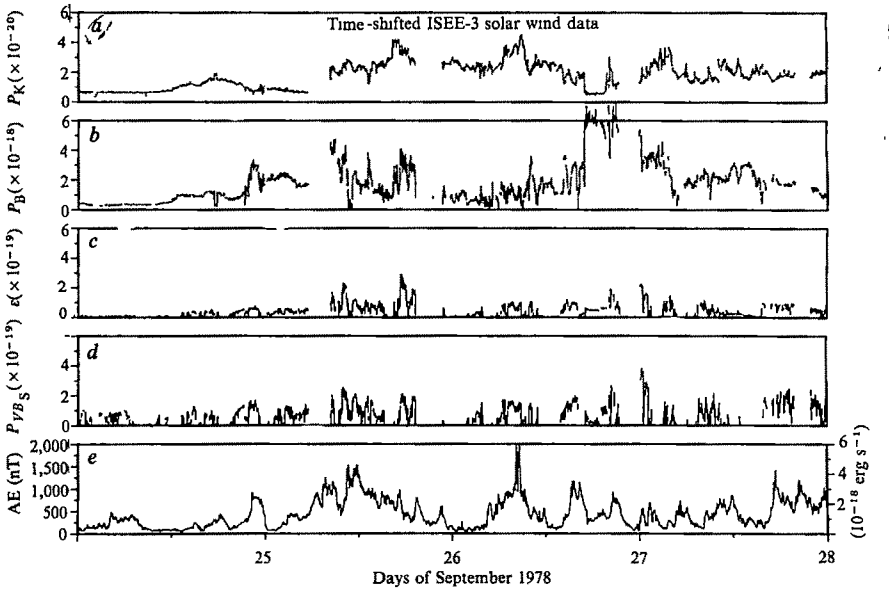


Fig. 1 *a–d*, Summaries of various power estimates (in erg s^{-1}) using data taken in September 1978 from the ISEE-3 spacecraft ~240 R_E from Earth in the upstream solar wind. These data have been time-shifted to account for propagation time delay to the Earth. *a*, P_K ($=\pi l_0^2 \rho V^3$) plotted for a several-day interval. *b*, The solar wind magnetic energy power P_B ($=\pi l_0^2 V(B^2/8\pi)$). *c*, *d*, Popular refinements of the magnetic energy power parameter: *c* shows ε ($=VB^2 \sin^4(\theta/2)(l_0^*)^2$, where $l_0^* = 7R_E$ and θ is the angle between the Earth's field and the interplanetary field; *d* shows VB_s ($=-B_z$ for negative IMF, and 0 for positive IMF) scaled to the Chapman-Ferrary interaction area. *e*, The Auroral electrojet[2] index, AE (scale to the left). Using empirical relationships[2,3] it is also possible to make magnetospheric energy dissipation estimates from AE (scale to the right).

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1. Desch, M. D. & Kaiser, M. L. *Nature* **310**, 755–757 (1984).
2 Baker, D. N *et al. J geophys. Res.* **88**, 6230–6242 (1983)
3 Akasofu, S.-I *Space Sci. Rev* **28**, 121–190 (1981).
4. Baker, D. N., Hones, E. W., Payne, J B & Feldman, W C. *Geophys Res. Lett* **8**, 179–182 (1981).
5. Voots, G R., Gurnett, D A. & Akasofu, S -I *J geophys Res* **82**, 2259–2266 (1977)
6. Gallagher, D. L. & D'Angelo, N. *Geophys Res Lett.* **8**, 1087–1089 (1981).
7. Crooker, N. U., Feynman, J. & J. T. Gosling *J geophys Res.* **82**, 1933–1937 (1977).
8 Baker, D. N. *Am. Inst. Phys. Conf Proc. Ser. Magnetospheric Phenomena in Astrophysics* (eds Epstein, R., and Feldman, W.) (AIP, New York, in the press)

DESCH AND KAISER REPLY—Baker and Bargatze have argued that high time resolution comparisons of auroral electrojet (AE) with the solar wind input lead to a much higher efficiency for the conversion of solar wind power into auroral kilometric radiation (AKR) than that estimated by us[1]. Therefore, if different efficiencies apply at Jupiter and Saturn, where physical processes are not as well understood, then our 'radiometric Bode's law' cannot be reliably extrapolated to the case of Uranus.

We agree that high time resolution studies have led to a better understanding of solar wind-magnetosphere coupling processes at the Earth than presently exists at either Jupiter or Saturn, but it is not clear that this understanding extends to solar wind-AKR coupling, nor is it apparent that conclusions drawn from longer-term averaging of the data are necessarily improper. Regarding the first point, Voots *et al.*[2] actually noted a rather small (51%) degree of correlation between AE and AKR, attributing this, in part, to the 'poor short timescale (< 1 h) correlation' that they observed. Thus, although the relationship between substorm activity (as indicated by AE) and the solar wind may be well understood, that between AKR power and the solar wind is not.

We used the solar wind kinetic energy flux, ρV^3 (where ρ is the solar wind mass density and V the solar wind speed), to characterize the energy input to each magnetosphere, because the radio emissions of the known radio planets show by far the best correlation with solar wind plasma properties, such as ρ or V, and little or no correlation with magnetic properties such as VB^2 (refs 3–5). For example, there is almost a one-to-one correspondence between the radio emission output of the AKR and the solar wind speed[3] and between the radio output of Saturn and the solar wind density[4]. Each of the studies of Desch and Kaiser has in common the consistent use of one-rotation-averaged solar wind and radio emission quantities. Certainly details of the interaction mechanism are lost when such long-term averages are used, but some coarse information about the interaction probably remains.

Our radiometric Bode's law is intended as a heuristic demonstration of one property of radio-emitting magnetospheres, namely, their gross radio efficiency. Whether or not this law reflects some underlying physical property of radio planets in general is not known; indeed it was not our intention to suggest that such a property exists. However, the potential predictive value of the proposed relationship is, we feel, of some interest and will be tested further by Voyager spacecraft observations of Uranus in the coming year.

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1. Desch, M. D & Kaiser, M L *Nature* **310**, 755–757 (1984).
2. Voots, G. R., Gurnett, D A. & Akasofu, S -I. *J geophys Res* **82**, 2259–2266 (1977)
3. Gallagher, D L & D'Angelo, N *Geophys Res. Lett.* **8**, 1087–1089 (1981)
4. Desch, M D & Rucker, H O *J. geophys Res* **88**, 8999–9006 (1983)
5 Desch, M D & Barrow, C. H *J geophys Res* **89**, 6819–6823 (1984)

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BIOGEN

RESEARCH SCIENTIST

The Massachusetts Institute of Technology (MIT), Whitaker College of Health Sciences, Technology and Management, announces an opening for a Research Scientist to work within the Center for Biological Information Processing integrating theoretical and experimental brain sciences. Responsibilities will include developing computer models of single neurons, dendritic trees and synapses from physiological and anatomical data, as well as interaction with visual psychophysics and the computational theories of vision. He or she will also explore computational properties of biophysical mechanisms and will apply these results to the analysis of the neural circuitries involved in the perception of motion and stereo

A Ph.D. in mathematics, physics or computer science is required. Experience in how computational theories can be implemented in algorithms, and how the underlying biophysical hardware can determine the nature of the algorithm is also required. A solid background in mathematics, knowledge of invertebrate and vertebrate biophysics, physiology and extensive computer experience is expected. LISP computer language desirable.

Interested applicants should send two copies of cover letter and curriculum vitae, as well as any relevant publications to: Dr. Tomaso Poggio, c/o MIT Personnel Office (E19-239), 77 Massachusetts Avenue, Cambridge, MA 02139. Reference Job #R84-573. MIT is an Affirmative Action/Equal Opportunity Employer.

Qualified minorities and women are encouraged to apply.

(NW1695)A

MIT

PLANT BREEDING INSTITUTE

Applications are invited for the following positions to work for a period of three years with a large, well-established team of molecular geneticists under the leadership of Dr R B Flavell:

1. POSTDOCTORAL FELLOW to further understanding of photosynthesis and chloroplast biochemistry by the insertion of new enzymes and genes into the chloroplasts of whole plants. The project involves the isolation, characterisation and reconstruction of genes, their insertion into plants using *Agrobacterium* vectors and plant tissue culture and biochemical and physiological assessments of the altered plants.
Candidates should have a first or upper second class honours degree in an appropriate biological subject with at least two years' experience in biochemistry or molecular biology, and a PhD. **Ref: CYT/205.**

2. SCIENTIFIC OFFICER to assist as above.
Candidates should have a first or upper second class honours degree in an appropriate biological subject. Experience in biochemistry or molecular biology would be an advantage. **Ref: CYT/206.**

3. POSTDOCTORAL FELLOW to develop transposable element genetics in specific dicot plants. The approach will include the insertion of already defined transposable elements from other species into the dicot crop species using *Agrobacterium* vectors and tissue culture. The project will exploit many of the techniques of molecular biology.
Candidates should have a first or upper second class honours degree in an appropriate biological subject with at least two years' experience in biochemistry, genetics or molecular biology, and a PhD. **Ref: CYT/207.**

4. SCIENTIFIC OFFICER to assist as above.
Candidates should have a first or upper second class honours degree in an appropriate biological subject. Experience in biochemistry, genetics or molecular biology would be an advantage. **Ref: CYT/208.**

Appointment for the Postdoctoral positions will be at Higher Scientific Officer (£7,435 to £10,039 pa), and at Scientific Officer for positions (2) and (4) (£5,909 to £8,153 pa). A non-contributory pension scheme is in operation.

Applications with curriculum vitae and names and addresses of three referees for the HSO grade and two for the SO grade should be sent to the Establishment Officer, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, *quoting the appropriate reference number,* by 23 May 1985.

The Institute is an Equal Opportunities Employer. (6241)A

QUEENSLAND INSTITUTE OF MEDICAL RESEARCH POSITIONS IN MOLECULAR BIOLOGY

The Queensland Institute of Medical Research, affiliated with the University of Queensland, is a major Australian Medical Research Centre It is situated on the medical campus of the University in association with the Royal Brisbane Hospitals complex. Research is broadly based in cancer and cell biology, molecular biology and parasitology, virology and immunology, epidemiology and public health There are extnesive national and international collaborative programmes.

The Institute is expanding its existing base in molecular biology, particularly in the recombinant DNA unit. Present work includes molecular mechanisms of genetic arrangement relating to gene activation, expression and transposition in eukaryotes, human gene isolation/structure, viral gene structure/control, parasite gene structural organization, together with special programmes directed to vaccine development against malaria parasites and dengue viruses. This expansion is made possible by new laboratory space and equipment.

Applications are called for two positions·

1. Molecular biologist/biochemist to work as a senior member of the recombinant DNA unit, contributing to ongoing programmes from a base of fundamental cloning and related techniques. The applicant would be encouraged to sustain independent research in this area. This position is funded from established (trust) funds, with salary according to qualifications and experience, in the range 24,840 to 38,847 Aust dollars p.a. Appointment will be for 5 years initially, but renewable.

2. Biochemist/Geneticist to work with the recombinant DNA unit and the human genetics laboratory, with particular reference to human gene isolation/structure, recombination mechanisms and their control in eukaryotes. The position is funded by a block grant from the Australian National Health and Medical Research Council, with salary according to qualifications and experience, in the range 21,411 to 32,634 Aust dollars p.a. Appointment will be up to 5 years.

Applicants should forward a full curriculum vitae, together with an outline of research interests with names and addresses of 3 referees to:

The Director
Queensland Institute of Medical Research
Bramston Terrace
Herston
Brisbane 4006 Australia
Phone: (07) 2536202
Telex: QIMRBR 145420

Applications close on 1 June, 1985. (W1822)A

UNIVERSITY OF CAPE TOWN

ASSOCIATE PROFESSOR/SENIOR LECTURER/ LECTURER IN BIOCHEMISTRY (MOLECULAR GENETICS AND MOLECULAR BIOLOGY)

Applications are invited for the above posts in the Department of Biochemistry. Candidates should have strong research interest and achievements in eucaryotic molecular genetics and experience in recombinant DNA technology Alternatively, candidates experienced in the field of structural investigation on proteins and protein-nucleic acid complexes will be considered. The posts may also be filled at the request of the applicant on a contractual basis for 2-3 years with either the possibility of renewal or conversion into a tenured post. Appointments according to qualifications and experience will be made on the salary scales:

Associate Professor	—	R28 889 - R35 377 p.a.
Senior Lecturer	—	R26 403 - R32 763 p.a.
Lecturer	—	R17 267 - R28 889 p.a.

with an annual bonus and attractive staff benefits.

Applicants should submit a full curriculum vitae, including the names and addresses of three referees not later than 7 June 1985, to the Registrar (Attention Appointments Office Ref. AC/5412) University of Cape Town, Rondebosch, 7700, South Africa Further information may be obtained from the Registrar or The Secretary, SA Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE. The University's policy is not to discriminate on the grounds of sex, race or religion Further information on the implementation of this policy is obtainable on request. (W1812)A

INSTITUTE OF TERRESTRIAL ECOLOGY

RADIOCHEMIST

A radiochemist is needed to join a small team, looking into the distribution of radionuclides in the environment. The appointee will be based at the Institute's Merlewood Research Station, Grange-Over-Sands, Cumbria.

Qualifications and Experience: The minimum qualification for the post is a good degree or equivalent. Previous experience is desirable.

Salary: The appointment will be to Higher Scientific Officer/Senior Scientific Officer grade with a salary scale of £7,435 - £10,039 pa for HSO or £9,329 - £12,050 pa for SSO. Starting salary will be according to age, qualifications and experience.

The Natural Environment Research Council is not a government department but conditions of service are similar to those of the Civil Service.

Application forms and further details are available from: Institute of Terrestrial Ecology, Administrative Headquarters, Monks Wood Experimental Station, Abbots Ripton, Huntingdon PE17 2LS Telephone 04873 381-8.

Please quote reference number: 229.

The closing date for applications is 9 May 1985.

NATURAL ENVIRONMENT RESEARCH COUNCIL

(6228)A